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The American Phytopathological Society

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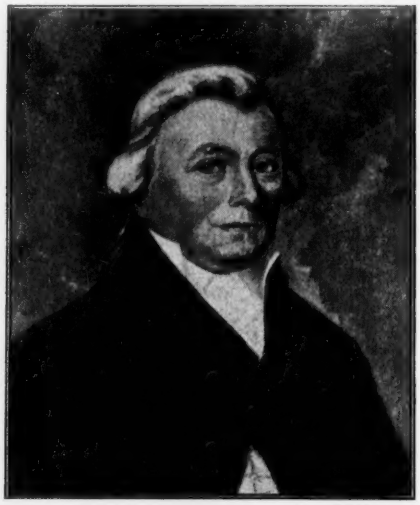

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EMIL GODFRED ARZBERGER

PHYTOPATHOLOGY

VOLUME 21 NUMBER 4

APRIL, 1931

EMIL GODFRED ARZBERGER

1877–1930

A. B. Stout

Emil Godfred Arzberger was born at Helenville, Wisconsin, on October 3, 1877. He died from endocarditis at Clarendon, Virginia, on January 29, 1930. His body rests in the cemetery at Helenville, Wisconsin, close to the scenes of his childhood. Of the immediate family a father, three brothers, and four sisters are still living.

For his early education Mr. Arzberger attended the public schools with graduation from the High School of Jefferson, Wisconsin, in 1897. He attended the State Normal School at Whitewater, Wisconsin, graduating there in 1903, after which he was principal of a graded school in Wisconsin for two years. He then entered the University of Wisconsin, completed the work for the degree of Ph.B. in 1906, and remained for three years as graduate student and assistant in botany. During the academic year of 1909–10 he was a Fellow at the Missouri Botanical Garden in St. Louis, Missouri, and at the end of this year he received the degree of M.A. from Washington University. The thesis for this degree was entitled "The fungous root-tubercles of *Ceanothus americanus*, *Elaeagnus argentea*, and *Myrica cerifera*," and it was published in the Twenty-first Annual Report of the Missouri Botanical Garden. From 1910 until 1913 he was assistant botanist of the Ohio State Experiment Station with principal work in pathology. For several months during the summer of 1913 he held a Research Scholarship at The New York Botanical Garden and from this he went to the Office of Nematology as assistant pathologist, and this position he held until his death.

Mr. Arzberger's interests in botany centered in pathology and mycology. Most of his research was devoted to organisms that inhabit the roots of plants and to mycorrhizae. He obtained in pure cultures various of the fungi concerned in the formation of mycorrhizae, he made careful cytological studies of these structures, and he was concerned in determining the relation of their presence to the growth which plants made. He was widely acquainted with the literature in this field and was able and critical in the consideration of it.

By natural inclination, Mr. Arzberger was of a quiet and retiring disposition. His friendships were deep and lasting. Those who knew him recognized and admired his integrity, sincerity, and worth, and were impressed with his knowledge, skill, and ability in the field of botanical science which he loved.

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PRELIMINARY EXPERIMENTS ON THE CONTROL OF CEREAL RUSTS BY KOLO DUST[1] [2]

W. C. BROADFOOT[3]

INTRODUCTION

In 1924 Kightlinger (5) reported "phenomenal control" of rusts on oats in New York by dusting with sulphur. Since that time considerable emphasis has been placed on and impetus given to sulphur dusting as a method for the control of cereal rusts. In 1926 Kightlinger and Whetzel (6) in New York, Bailey and Greaney (1) in Manitoba, and Lambert and Stakman (7) in Minnesota obtained evidence in support of Kightlinger's earlier statement that sulphur controls rust under certain conditions. In 1927 Bailey and Greaney (2) reported the results of trials with horse-drawn traction dusters as well as those of small-plot experiments. They stated, however, that stem rust was such a negligible factor in Manitoba that year that conditions did not favor testing the effectiveness of sulphur dust under field conditions. Bailey and Greaney (3), in 1928, reported favorable results in some instances from dusting under field conditions both by horse-drawn dusters and by aeroplane. Greaney (4) also reported that the fineness of the particles, humidity, and temperature greatly influenced the toxicity and fungicidal effectiveness of the dust.

Preliminary experiments also had been made in Minnesota. It had been found by Lambert and Stakman (7) that Kolo dust, as well as certain other dusts, would effectively prevent stem rust provided the applications were made at the proper time and with the right kind of dust. Not only was the rust prevented, but grain yields were considerably increased. Very little is known, however, about the number of applications that would be necessary, the time of application, and the effect of dusting in different

[1] The experiments were made cooperatively between the Niagara Sprayer Company and the University of Minnesota, under a special grant made by the Niagara Sprayer Company to the University.

[2] Published with the approval of the Director as Paper No. 962 of the Journal Series of the Minnesota Agricultural Experiment Station.

[3] The writer here expresses his appreciation to Dr. E. C. Stakman, Professor of Plant Pathology, University of Minnesota, and Pathologist, Bureau of Plant Industry, United States Department of Agriculture, for his helpful advice and criticism; to Dr. E. B. Lambert, Associate Pathologist, Bureau of Plant Industry, United States Department of Agriculture, for his helpful advice in outlining the project; to Mr. C. V. Kightlinger, formerly Agent, Bureau of Plant Industry, United States Department of Agriculture, for his assistance in taking notes at St. Paul, Morris, and Crookston, Minnesota; to Mr. R. S. Dunham, Agronomist at the N. W. School and Station, Crookston, Minnesota; and to Mr. R. O. Bridgford, Agronomist at the West Central School and Station, Morris, Minnesota, for their cooperation and assistance at their respective stations.

seasons. The writer's experiments were made in order to obtain additional and more definite information on some of these questions.

Specifically, the objects of the writer's experiments were to determine, in a preliminary way, the most effective and practicable number, time, rate, and method of application of Kolo sulphur dust for the control of stem and leaf rusts and the subsequent effect on yield, bushel weight, grade, protein content, and the setting of seed of wheat. Experiments were conducted in 1927 at Morris, Crookston, and St. Paul, Minnesota.

MATERIALS AND METHODS

Varieties of wheat most extensively grown in Minnesota were used in these tests. Marquis and Ruby, common bread wheats, and Mindum, a durum wheat, were sown with an 8-foot nursery seeder at a uniform rate of approximately 90 pounds an acre. When the young plants were about 12 inches high, the seeded areas were divided into plots 8 feet square, separated from one another by 1-foot alleys. More than 2,000 plots were included in the experiment.

Kolo dust, a colloidal sulphur dust, supplied by the Niagara Sprayer Company of Middleport, N. Y., was used throughout the experiments. The dust was applied by means of a hand duster (Niagara Blower Gun No. 42, at Morris and St. Paul, and a Root Hand Gun, at Crookston). The hand dusters were calibrated so that 10, 20, 30, and 40 revolutions of the crank, made at the rate of one revolution a step or pace in walking around the plot, discharged dust at the rates of 15, 30, 45, or 60 pounds per acre per application, respectively. By the expressions 15-, 30-, 45-, or 60-pound rate, used hereafter in this paper, is meant the amount of dust used per acre per application. The dusters were checked at frequent intervals throughout the experiments to insure reasonable accuracy and uniformity in the rates of application.

The experiments were made on the following soil types: Hempsted silt loam at St. Paul, Clarion silt loam at Morris, and Fargo clay loam at Crookston. The soil in the plots at each of these places appeared to be fairly homogeneous.

Stem-rust data were obtained by making estimates of the average amount of rust on the plants in each plot according to the scale adopted by the Office of Cereal Crops and Diseases, United States Department of Agriculture. Separate notes were taken on the amount of rust on the "neck" or peduncle, sheath or boot, and the lowest internode.

As there appeared to be no significant and consistent differences in the amount of rust on the sheaths or boots and on the lowest internodes of plants in the various plots, only the data on severity of rust infection on the peduncle will be given. It is the peduncle of the plant that we are chiefly interested in protecting for practical purposes, since stem rust causes

greatest damage on this part of the plant. Rust usually is already present on the sheath or boot and on the lowest internode, when practical dusting operations ordinarily would be started, and dusting can not be expected to control rust on those parts of the plant already infected when dusting is begun. Therefore, the importance of protecting the peduncle seems paramount.

When the wheat was ripe, square-yard samples were harvested from each plot and later threshed with a nursery thresher. The seed from each plot was weighed and the yield in bushels per acre was then computed. The difference in yield might have been greater if an ordinary threshing machine had been used. Undoubtedly more of the light, shrunken seed from the nondusted plots would have been blown into the straw stack had the grain been threshed with a field machine.

Determinations of the weight per bushel of wheat from each plot were made by the methods recommended by the United States Grain Standards Act, except that, since the samples were small, a one-half-pint tester was used instead of the regular one-quart tester.

The samples were graded according to the official grain standards recommended by the United States Grain Standards Act.

From the data on the yield of wheat from the nondusted plots at the various localities, a probable error for each experiment was calculated by the use of the following formula: $P.E. = \pm .6745 \frac{Ef.d^2}{n} \over N$ where d = deviation in bushels of a class from the mean, f = frequency, n = total number of individuals, and N = the number of replications. The following probable errors were obtained for Marquis wheat:

Locality	Variety	P. E. in per cent
Morris	Marquis	5.3087
St. Paul	" (early planting)	5.8386
"	" (late planting)	9.9930
Crookston	"	7.1233

The average probable error for Morris, Crookston, and St. Paul = $1/3\sqrt{5.3^2 + 5.8^2 + 7.1^2} = 3.53\%$.

The average probable error for Crookston and St. Paul (whenever Morris data were not obtained) = $1/2\sqrt{5.3^2 + 7.1^2} = 4.58\%$.

Random samples of seed from the plots dusted for the control of leaf rust were analyzed for protein content by the Kjeldahl method for nitrogen analysis.

For the schedule consisting of three applications of Kolo dust at the four different rates there are six different times of application, as follows:

First Application	*Second Application*	*Third Application*
1. Flowering time	4 days after first	4 days after second
2. " "	6 " " "	" " " "
3. " "	8 " " "	" " " "
4. 5 days after flowering time	4 " " "	" " " "
5. " " " " "	6 " " "	" " " "
6. " " " " "	8 " " "	" " " "

For the schedule consisting of two applications of Kolo dust at the four different rates, there are eight different times of application, as follows:

First Application	*Second Application*
1. Flowering time	4 days after first
2. " "	6 " " "
3. " "	8 " " "
4. " "	10 " " "
5. 5 days after flowering time	4 " " "
6. " " " " "	6 " " "
7. " " " " "	8 " " "
8. " " " " "	10 " " "

For the schedule consisting of one application of Kolo dust at the four different rates, there are four different times of application, as follows:

Treatments	*Time of Application*
First	At flowering time
Second	5 days after flowering time
Third	10 " " " "
Fourth	15 " " " "

The fact that the stem-rust epiphytotic was very severe at Morris in 1927 afforded a good opportunity to test the effectiveness of dusting with Kolo dust. The rust developed rapidly, especially during the latter part of the growing season, and, together with hot, dry weather, decreased yield and quality of wheat in this district very materially. At Crookston, about 200 miles north of Morris, the epiphytotic was not so destructive. The development of rust was checked to a considerable extent by cool weather during the first two weeks in August. At St. Paul, about 150 miles east and slightly south of Morris, the epiphytotic was almost as severe as at Morris, although grain probably was damaged less by hot dry weather.

Marquis wheat was sown at Morris on April 25, at Crookston on April 30, and at St. Paul on May 2. The wheat plants flowered rather uniformly;

at Morris on July 6, at Crookston July 11, and at St. Paul on July 8. Flowering-time applications were made on these dates. Rust notes were taken at Morris on August 1, at Crookston on August 12, and at St. Paul on August 2. The plots were harvested at Morris on August 9, at Crookston on August 19 to 23, and at St. Paul on August 14.

THE EFFECT OF THREE APPLICATIONS OF KOLO DUST ON MARQUIS WHEAT IN 1927

The mean average percentages of stem rust on the peduncle of the wheat plant were significantly lower on the dusted than on the nondusted plots at the three stations (Table 1). Dusting controlled rust so well at Morris that the dusted plots easily could be singled out from a distance of 100 feet. The bright and clean appearance of the plants in the dusted plots contrasted sharply with the dull, dark, dead appearance of those in the nondusted plots. The mean average percentage of rust in the nondusted plots ranged from 60.9 to 68.3 in comparison with 13.0 to 41.3 in the dusted plots. As a rule, the mean average percentage was lower in those plots dusted first at flowering time than in those in which the first application was made 5 days later. It should be pointed out that the results of making the first application at flowering time were better in Crookston than at Morris and St. Paul, although the exact reason is not known.

The best schedule at the three stations, from the standpoint of time of application, was the F.T. + 6 or 8 + 4, which means that the first application was made at flowering time, the second either 6 or 8 days after the first, and the third 4 days after the second. For the F.T. + 6 + 4 schedule the mean average percentages of rust on the plots dusted at the 15-, 30-, 45-, and 60-pound rates were 26.1, 21.9, 18.8, and 14.0 per cent, respectively (Table 1, Plots 56, 63, 70, and 77). For the F.T. + 8 + 4 schedule at the 15-, 30-, 45-, and 60-pound rates, the mean average percentages were 23.6, 17.6, 15.4, and 13.0 per cent, respectively (Table 1, Plots 57, 64, 71, and 78). From this it is evident that the 60-pound rate was considerably more effective than the 15-pound rate but probably not significantly more effective than the 30- and 45-pound rates. It also seems that there was no significant difference in the mean average percentage of rust in plots dusted according to the F.T. + 6 + 4 and F.T. + 8 + 4 schedules.

The mean average yields of wheat were uniformly and significantly higher on the dusted than on the nondusted plots at the three stations. For the near-by nondusted plots the mean average yields were 24.57 ± 0.87, 25.16 ± 0.89, 24.33 ± 0.86, 21.48 ± 0.76, and 23.26 ± 0.82 bushels per acre, respectively (Table 1, Plots 54, 61, 68, 75, and 82). The average yield for all the nondusted plots at the three stations, 120 in all, was 23.31 bushels per acre. The plots on which the first application was made at flowering

TABLE 1.—*The effect of 3 applications of Kolo dust applied at 15–, 30–, 45–, and 60-pound rates and at various intervals in relation to flowering time of wheat plant on the control of stem rust, increase in yield, weight per bushel, and grade of Marquis wheat at Morris, Crookston, and St. Paul, Minnesota, in 1927*

Plot No.	Application		Average[b] percentage of stem rust on peduncle				Average[b] yield in bushels per acre				Average[b] weight in pounds per bushel				Average[b] grade number			
	Rate in pounds per acre	Time[a]	M	C	St. P	Mean av.	M	C	St. P	Mean average	M	C	St. P	Mean av.	M	C	St. P	Mean av.
54	0	Check	77.5	72.5	55.0	68.3	19.91	28.54	25.25	24.57 ± 0.87	48.5	53.8	51.3	51.2	S. G.[c]	4	5	5
55	15	FT + 4 + 4	25.0	67.5	28.8	40.4	27.47	30.67	28.63	28.92 ± 1.02	54.9	55.4	53.8	54.7	3	3	4	3
56	15	" + 6 + 4	20.8	35.0	22.5	26.1	27.43	36.67	30.76	31.62 ± 1.12	55.4	56.0	54.5	55.3	3	3	3	3
57	15	" + 8 + 4	10.8	35.0	25.0	23.6	28.05	35.65	30.72	31.47 ± 1.11	56.9	56.5	54.5	56.0	2	2	3	3
58	15	(FT + 5) + 4 + 4	31.3	18.8	36.3	28.8	26.14	40.14	28.96	31.75 ± 1.12	54.0	58.9	53.3	55.4	4	1	4	3
59	15	" + 6 + 4	36.3	25.0	41.3	34.2	24.41	39.16	27.07	30.21 ± 1.07	54.4	56.8	52.5	54.6	4	2	4	3
60	15	" + 8 + 4	43.8	46.3	38.8	41.3	23.47	42.23	26.40	30.70 ± 1.08	53.5	56.0	53.0	54.2	4	3	4	4
61	0	Check	81.3	68.8	47.5	65.9	20.45	29.96	25.07	25.16 ± 0.89	49.0	55.8	49.3	51.4	S. G.	3	S. G.	5
62	30	FT + 4 + 4	23.8	45.0	30.0	32.9	24.49	35.03	31.52	30.35 ± 1.07	55.5	57.4	52.5	55.1	3	2	4	3
63	30	" + 6 + 4	17.0	22.5	26.3	21.9	25.87	40.45	32.81	33.04 ± 1.17	55.5	57.7	55.0	56.1	3	1	3	3
64	30	" + 8 + 4	11.5	20.0	21.3	17.6	30.72	40.41	31.92	34.35 ± 1.21	56.4	58.0	54.3	56.2	3	1	4	3
65	30	(FT + 5) + 4 + 4	33.8	7.0	21.3	20.7	24.23	38.20	29.16	30.53 ± 1.08	54.6	59.0	52.5	55.4	3	1	4	3
66	30	" + 6 + 4	36.3	14.3	35.0	28.5	22.98	35.92	25.56	28.15 ± 0.99	53.3	57.8	53.5	54.9	4	1	4	3
67	30	" + 8 + 4	50.0	41.3	18.8	36.7	21.78	31.92	29.72	27.81 ± 0.98	51.9	56.6	54.0	54.2	5	2	4	4
68	0	Check	81.3	82.5	38.8	67.9	21.02	32.45	19.52	24.33 ± 0.86	47.4	54.3	48.3	50.0	S. G.	4	S. G.	5
69	45	FT + 4 + 4	27.5	55.0	21.3	34.6	27.83	42.23	26.63	32.23 ± 1.14	54.8	57.5	52.5	54.9	3	1	4	3
70	45	" + 6 + 4	21.3	18.8	16.3	18.8	27.92	43.52	29.61	33.68 ± 1.19	56.0	58.4	54.3	56.2	3	1	4	3
71	45	" + 8 + 4	13.8	17.5	15.0	15.4	30.36	36.36	24.63	30.45 ± 1.08	57.1	57.5	52.8	55.8	2	1	4	3
72	45	(FT + 5) + 4 + 4	30.0	10.0	18.8	19.6	26.18	32.45	25.87	28.17 ± 0.99	54.3	56.9	52.3	54.5	4	2	5	3
73	45	" + 6 + 4	31.3	17.5	22.5	23.8	27.78	29.87	25.74	27.80 ± 0.98	54.3	57.4	53.0	54.9	4	2	4	3
74	45	" + 8 + 4	45.0	43.8	20.0	36.3	24.32	36.03	27.78	29.38 ± 1.04	53.2	56.6	54.5	54.8	4	2	3	3
75	0	Check	82.5	63.8	36.3	60.9	17.47	25.29	22.67	21.48 ± 0.76	47.2	51.8	51.0	50.0	S. G.	5	5	5
76	60	FT + 4 + 4	27.5	48.8	21.3	32.5	30.14	31.61	33.61	31.79 ± 1.12	55.9	56.0	55.8	55.9	3	3	3	3
77	60	" + 6 + 4	22.5	10.8	8.8	14.0	32.18	44.54	32.09	36.27 ± 1.28	55.0	57.6	58.8	57.1	3	1	1	2
78	60	" + 8 + 4	13.8	13.8	11.3	13.0	33.47	38.41	30.67	34.18 ± 1.21	57.1	56.3	58.0	57.1	2	3	1	2
79	60	(FT + 5) + 4 + 4	35.0	11.3	17.5	21.3	27.74	39.39	31.21	32.78 ± 1.16	53.0	56.0	55.0	54.7	4	3	3	3
80	60	" + 6 + 4	30.0	15.8	22.5	22.8	26.19	36.67	28.67	30.51 ± 1.08	54.9	53.9	52.5	53.8	3	4	4	4
81	60	" + 8 + 4	43.8	28.8	18.8	30.5	24.07	34.94	29.79	29.80 ± 1.05	51.1	55.3	54.8	53.7	5	3	3	4
82	0	Check	85.0	61.3	36.3	60.9	18.27	28.85	22.67	23.26 ± 0.82	48.1	54.9	51.0	51.3	S. G.	3	5	5

[a] FT + 4 + 4 = First application at flowering time, the second 4 days after the first, and the third 4 days after the second.
" + 6 + 4 = " " " " " " 6 " " " " " " " 4 " " " "
" + 8 + 4 = " " " " " " 8 " " " " " " " 4 " " " "
(FT + 5) + 4 + 4 = " " 5 days after " " " 8 " " " " " " " 4 " " " "
(FT + 5) + 6 + 4 = " " 5 days after " " " 8 " " " " " " " 4 " " " "
(FT + 5) + 8 + 4 = " " 5 days after " " " 8 " " " " " " " 4 " " " "

[b] Average of four systematically distributed plots at each locality.

[c] S. G. = Sample Grade.

time, in addition to being freer from stem rust, yielded consistently more wheat than those plots on which the first application was made 5 days after flowering time. For the F.T. + 6 + 4 schedule the mean average yields of wheat from these plots dusted at the 15-, 30-, 45-, and 60-pound rates were 31.62 ± 1.12, 33.04 ± 1.17, 33.68 ± 1.19, and 36.27 ± 1.28 bushels per acre, respectively (Table 1, Plots 56, 63, 70, and 77). These yields, when compared with the average yield of 23.31 bushels per acre from the 120 nondusted plots, represent an increase of 8.31, 9.73, 10.37, and 12.96 bushels per acre, or increases of 35.6, 41.7, 44.5, and 55.6 per cent, respectively. For the F.T. + 8 + 4 schedule the mean average yields for the plots dusted at the 15-, 30-, 45-, and 60-pound rates were 31.47 ± 1.11, 34.35 ± 1.21, 30.45 ± 1.08, and 34.18 ± 1.21 bushels per acre, respectively (Table 1, Plots 57, 64, 71, and 78). These yields, when compared with those of the nondusted plots, represent an increase of 8.16, 11.04, 7.14, and 10.87 bushels per acre, or increases of 35.0, 47.4, 30.6, and 46.6 per cent, respectively. The highest yielding plots, which were dusted three times at Morris, Crookston, and St. Paul, when considered separately, yielded 76, 52, and 44 per cent more than the near-by nondusted plots, respectively. From these data it is seen that the 15- and 30-pound rates were almost as effective as the 45- and 60-pound rates. This again emphasizes the fact that timeliness of application is one of the most important factors to be considered. At Crookston, when the first application was made 5 days after flowering time, the yield was almost as high as when the first application was made at flowering time, but this was not true at Morris and St. Paul.

The mean average weights per bushel and grades of wheat were uniformly and significantly higher for the dusted than for the nondusted plots at the three stations. For the near-by nondusted plots the mean average weights were 51.2, 51.4, 50.0, 50.0, and 51.3 pounds, respectively (Table 1, Plots 54, 61, 68, 75, and 82), and the mean average grade for the wheat from each was No. 5. The average weight per bushel for the 120 nondusted plots at the three stations was 50.2 pounds, grading No. 5. For the F.T. + 6 + 4 schedule the mean average weights for the wheat from the plots dusted at the 15-, 30-, 45-, and 60-pound rates were 55.3, 56.1, 56.2, and 57.1 pounds, and on the average graded No. 3 for the first three rates and No. 2 for the last (Table 1, Plots 56, 63, 70, and 77). These weights, when compared with the average weight of 50.2 pounds for the 120 nondusted plots, represent increases in weight of 5.1, 5.9, 6.0, and 6.9 pounds per bushel, respectively, as well as an increase in grade from No. 5 to Nos. 3 and 2. For the F.T. + 8 + 4 schedule the mean average weights of wheat from the plots dusted at the 15-, 30-, 45-, and 60-pound rates were 56.0, 56.2, 55.8, and 57.1 pounds per bushel, grading the same as in the F.T. + 8 + 4 schedule (Table 1, Plots 57, 64, 71, and 78). These weights, when compared with 50.2 pounds, the average of 120 nondusted plots at the three

(FT + 5) + 6 + 4 = " " 5 days after " " " " " " " " " " " " " " " "
(FT + 5) + 8 + 4 = " " 5 days after " " " " " " " " " " " " " " " "
[a] Average of four systematically distributed plots at each locality. [c] S. G. = Sample Grade.

stations, represent increases in weight of 5.8, 6.0, 5.6, and 6.9 pounds per bushel, respectively, as well as an increase in grade from No. 5 to Nos. 3 and 2. As a rule, the weight per bushel was higher for the plots receiving the first application at flowering time than for those receiving it 5 days later. From these data it is evident that the 15-, 30-, and 45-pound rates were about equally effective, whereas the wheat from the plots dusted at the 60-pound rate weighed and graded uniformly slightly higher than that from those dusted at the lower rates. It is doubtful, however, whether the increase in weight per bushel and grade of wheat from plots receiving 3 applications at the 60-pound rate, 180 pounds in all, would pay for the additional dust and labor, especially since 3 applications at the 15-pound rate, 45 pounds in all, prove almost as effective.

THE EFFECT OF TWO APPLICATIONS OF KOLO DUST ON MARQUIS WHEAT

The mean average percentages of stem rust on the peduncle of the wheat plants were markedly lower on the dusted than on the nondusted plots at the three stations. The mean average percentages on the near-by nondusted plots ranged from 64.5 to 63.3, whereas on the dusted plots they ranged from 28.6 to 54.2. The differences in the mean average percentages of rust on the plots where the first application was made either at flowering time or 5 days later were not very great, although there appeared to be slightly less rust in the former case, especially at Morris and St. Paul. From the examination of table 2, it is apparent that there are no significant differences in the mean average percentages of rust on the plots dusted at the 15-, 30-, 45-, and 60-pound rates.

Since the amount of rust at Morris was the lowest in the plots dusted first at flowering time, followed by a second dusting 6 or 8 days later, only these plots were harvested along with all the plots dusted at the 60-pound rate. At Crookston and St. Paul all the plots dusted twice were harvested. The mean average yields were consistently higher on the dusted than on the nondusted plots. The mean average yields on the near-by nondusted plots compare favorably with the average of all the nondusted plots in the entire experiment. For the F.T. + 6 schedule the mean average yields for the plots dusted at the 15-, 30-, 45-, and 60-pound rates were, respectively, higher than those of the nondusted plots. For the F.T. + 8 schedule where the second application was made 8 instead of 6 days after the flowering-time application, the mean average yields for the plots dusted at the above rates showed increase of 30.0, 23.3, 39.7, and 29.6 per cent, respectively, in comparison with that of the nondusted plots. A careful examination of these data and the data given for the F.T. + 6 schedule shows that the 15- and 30-pound rates were practically as effective as the 45- and 60-pound rates, as measured by increases in yield. Further comparisons of the relative efficiency of the two applications when applied at the former rates are

TABLE 2.—*The effect of 2 applications of Kolo dust applied at 15-, 30-, 45-, and 60-pound rates and at various intervals in relation to flowering time of wheat plant on the control of stem rust, increase in yield, weight per bushel, and grade of Marquis wheat at Morris, Crookston, and St. Paul, Minnesota, in 1927*

Plot No.	Application		Average[b] percentage of stem rust on peduncle				Average[b] yield in bushels per acre				Average[b] weight in pounds per bushel				Average[b] grade number			
	Rate in pounds per acre	Time[a]	M.	C.	St. P	Mean av.	M	C	St. P	Mean average	M	C	St. P	Mean av.	M	C	St. P	Mean av.
18	0	Check	75.0	77.5	46.3	66.3	18.49	25.51	20.00	21.33 ± 0.75	45.9	54.8	48.5	49.7	S. G.[c]	3	S. G.	5
19	15	FT + 4	40.0	68.8	23.8	44.2	.....	27.11	24.60	25.86 ± 1.18	.....	56.0	50.3	53.2	.....	3	5	4
20	15	" + 6	32.5	62.5	18.8	37.9	28.58	35.07	26.63	30.09 ± 1.06	55.9	56.8	53.0	55.2	3	2	4	3
21	15	" + 8	28.8	68.8	22.5	40.0	25.29	36.41	29.24	30.31 ± 1.07	55.6	57.0	53.8	55.5	3	2	4	3
22	15	" + 10	42.5	55.0	25.0	40.8	.....	37.96	29.57	33.77 ± 1.55	.....	57.1	54.3	55.7	.....	2	4	3
23	30	" + 4	40.0	66.3	21.3	32.3	.....	36.23	30.36	22.20 ± 1.02	.....	55.9	55.3	55.6	.....	3	3	3
24	30	" + 6	31.3	67.5	16.3	38.4	29.14	31.03	34.05	31.41 ± 1.11	54.1	56.3	56.5	55.6	4	3	2	3
25	30	" + 8	32.5	56.3	26.3	38.4	28.19	27.74	30.27	28.73 ± 1.01	55.4	55.0	56.3	55.6	3	3	3	3
26	30	" + 10	41.3	17.0	27.5	28.6	.....	36.98	30.49	33.74 ± 1.55	.....	57.8	55.0	56.4	.....	1	3	3
27	0	Check	72.5	77.5	45.0	65.0	18.54	25.83	21.69	22.02 ± 0.78	47.1	54.1	49.3	50.2	S. G.	4	S. G.	5
28	45	FT + 4	42.5	76.3	43.8	54.2	.....	33.65	26.58	30.11 ± 1.38	.....	55.6	53.5	54.6	.....	3	4	3
29	45	" + 6	42.5	72.5	41.3	52.1	29.43	33.29	31.87	31.53 ± 1.11	55.3	56.3	55.3	55.6	3	3	3	3
30	45	" + 8	32.5	58.8	23.8	38.4	28.54	35.52	33.65	32.57 ± 1.15	56.3	55.1	53.5	55.0	3	3	4	3
31	45	" + 10	47.5	15.0	23.8	28.8	.....	38.01	32.50	35.26 ± 1.62	.....	56.6	54.8	55.7	.....	2	3	3
32	60	" + 4	43.8	50.0	38.8	44.2	24.41	28.72	29.47	27.53 ± 0.97	55.4	55.1	52.8	54.4	3	3	4	4
33	60	" + 6	30.0	56.3	28.8	38.4	25.70	31.52	31.60	29.61 ± 1.05	55.8	54.5	52.5	54.3	3	3	4	4
34	60	" + 8	31.3	41.3	26.3	32.6	23.61	33.87	33.14	30.21 ± 1.07	56.3	55.5	53.3	55.0	3	3	4	3
35	60	" + 10	45.0	25.0	35.0	35.0	20.67	38.45	30.38	29.50 ± 1.04	55.7	56.6	55.0	55.8	3	2	3	3
36	0	Check	72.5	72.5	48.8	64.6	16.86	28.00	25.60	23.49 ± 0.83	44.9	53.0	48.5	48.5	S. G.	4	S. G.	S. G.
37	15	(FT + 5) + 4	51.3	30.0	31.3	34.2	.....	34.14	25.16	29.65 ± 1.36	.....	55.6	51.5	53.6	.....	3	5	4
38	15	" + 6	51.3	43.8	40.0	45.0	.....	29.92	22.94	26.43 ± 1.21	.....	54.4	49.5	52.0	.....	4	5	5
39	15	" + 8	52.5	45.0	27.5	41.7	.....	26.45	26.00	26.23 ± 1.20	.....	53.5	51.3	52.4	.....	4	5	5
40	15	" + 10	53.8	65.0	33.8	50.9	.....	31.36	20.40	25.88 ± 1.19	.....	53.0	45.3	49.2	.....	4	S. G.	S. G.
41	30	" + 4	60.0	21.3	28.8	36.7	.....	43.79	22.71	33.25 ± 1.52	.....	56.0	49.8	52.9	.....	3	5	4
42	30	" + 6	60.0	35.0	28.8	41.3	.....	39.52	22.66	31.09 ± 1.42	.....	55.4	51.8	53.6	.....	3	5	4
43	30	" + 8	57.5	53.8	21.3	44.2	.....	35.25	23.78	29.52 ± 1.35	.....	55.3	51.0	53.2	.....	4	5	4
44	30	" + 10	62.5	62.5	26.3	50.4	.....	33.63	19.74	26.69 ± 1.22	.....	54.4	46.7	50.6	.....	4	S. G.	5
45	0	Check	77.5	77.5	38.8	64.6	18.98	27.07	22.14	22.73 ± 0.80	47.8	52.7	48.5	49.7	.....	4	S. G.	4
46	45	(FT + 5) + 4	55.0	13.8	25.0	31.3	.....	35.88	23.56	29.72 ± 1.36	.....	57.1	50.8	54.0	.....	2	5	4

TABLE 2.—*Continued*

Plot No.	Application		Average[b] percentage of stem rust on peduncle				Average[b] yield in bushels per acre				Average[b] weight in pounds per bushel				Average[b] grade number			
	Rate in pounds per acre	Time[a]	M	C	St. P	Mean av.	M	C	St. P	Mean average	M	C	St. P	Mean av.	M	C	St. P	Mean av.
47	45	(FT + 5) + 6	55.0	30.0	23.8	36.3	.....	38.23	24.54	31.39 ± 1.44	.....	56.5	50.3	53.4	.....	2	5	4
48	45	" + 8	50.0	51.3	15.0	38.8	.....	36.27	27.30	31.78 ± 1.46	.....	56.5	51.5	54.0	.....	2	5	4
49	45	" + 10	52.5	67.5	22.5	47.5	.....	29.38	24.40	26.89 ± 1.23	.....	54.3	52.3	53.3	.....	4	5	4
50	60	" + 4	52.5	18.8	18.8	30.0	22.32	35.47	30.41	29.40 ± 1.04	50.2	57.0	54.54	53.9	5	2	3	4
51	60	" + 6	51.3	28.8	21.3	33.8	23.21	39.16	25.60	29.32 ± 1.03	52.6	57.4	54.8	54.9	4	2	3	3
52	60	" + 8	47.5	46.3	23.8	39.2	23.16	35.78	31.38	30.11 ± 1.06	51.1	57.3	55.3	54.6	5	2	3	3
53	60	" + 10	50.0	63.8	35.0	49.6	23.82	28.72	29.92	27.49 ± 0.97	51.8	56.5	54.0	54.1	5	2	4	4
54	0	Check	77.5	72.5	55.0	68.3	19.91	28.54	25.25	24.57 ± 0.87	48.5	53.8	51.3	51.2	S. G.	4	5	5

[a] FT + 4 = First application at flowering time and the second 4 days after the first application.
" + 6 = " " " " " " 6 " " " " "
" + 8 = " " " " " " 8 " " " " "
" + 10 = " " " " " " 10 " " " " "
(FT + 5) + 4 = " " 5 days after " " " " 4 " " " " "
" + 5) + 6 = " " " " " " " " 6 " " " " "
" + 5) + 8 = " " " " " " " " 8 " " " " "
" + 5) + 10 = " " " " " " " " 10 " " " " "

[b] Average of four systematically distributed plots at each locality.
[c] S. G. = Sample Grade.

not justifiable, since the mean average yield data for the plots dusted on the other schedules do not include the Morris data, where the yields on all the plots were much lower than at either Crookston or St. Paul. This then would necessarily give higher mean average yields. The highest-yielding plots receiving two applications of Kolo dust at Morris, Crookston, and St. Paul, when considered separately, yielded 58, 38, and 51 per cent more than the near-by nondusted plots, respectively. These increases of 58, 38, and 51 per cent for the highest-yielding plots dusted twice at Morris, Crookston, and St. Paul compare favorably with the increases of 76, 52, and 44 per cent for the highest-yielding plots dusted three times at the same stations, although, with the exception of those at St. Paul, they are not so high (Table 4).

The mean average weights per bushel and the grades were higher for the dusted than for the nondusted plots at the three stations. The mean average weights for the near-by nondusted plots were 49.7, 50.2, 48.5, 49.7, and 51.2 pounds, respectively (Table 2, Plots 18, 27, 36, 45, and 54). The mean average grade of each near-by nondusted plot was No. 5. Wheat in the F.T. + 6 schedule averaged grade No. 3 for the 15-, 30-, and 45-pound rates and No. 4 for the 60-pound rate, while its mean average represented increases of 5.0, 5.4, 5.4, and 4.1 pound per bushel, respectively. For the F.T. + 8 schedule where the second application was made 8 instead of 6 days after the flowering-time application, the mean average weights represented an increase of 5.3, 5.4, 4.8, and 4.8 pounds, respectively, as well as an increase in grade from No. 5 up to a No. 3 in comparison with that of the nondusted plots. Further comparisons of the efficiency of two applications when applied on the other schedules are not justifiable since the mean average weight per bushel and grade do not include the Morris data, where the weight per bushel and grade were much lower than at Crookston and St. Paul. From the data presented it is evident, however, that two applications at the 15-, 30-, 45-, and 60-pound rate were about equally effective. It should be pointed out here that the increases in mean average weight per bushel and grade of wheat from plots receiving two applications of Kolo dust compare very favorably with those from the plots receiving three applications (Table 4).

THE EFFECT OF ONE APPLICATION OF KOLO DUST ON MARQUIS WHEAT

The mean average percentages of stem rust on the peduncle of the wheat plant (Table 3) were slightly lower on the plots receiving one application of Kolo dust than on the nondusted plots. However, the differences do not seem significant and they do not even constitute practical control. The mean average percentages of rust on the near-by nondusted plots were 71.3 and 66.3, respectively (Table 3, Plots 9 and 18). The mean average percentages on the plots dusted once ranged from 40.8 to 62.1. The differ-

TABLE 3.—*The effect of 1 application of Kolo dust applied at 15-, 30-, 45-, and 60-pound rates and at various intervals in relation to flowering time of wheat plants on the control of stem rust, increase in yield, weight per bushel, and grade of Marquis wheat at Morris, Crookston, and St. Paul, Minnesota, in 1927*

Plot No.	Application		Average[b] percentage of stem rust on peduncle				Average[b] yield in bushels per acre				Average[b] weight in pounds per bushel				Average[b] grade number			
	Rate in pounds per acre	Time[a]	M	C	St. P	Mean av.	M	C	St. P	Mean average	M	C	St. P	Mean av.	M	C	St. P	Mean av.
1	15	FT	51.3	78.8	37.5	55.9	.....	35.96	27.73	31.85 ± 1.46	.....	56.5	51.3	53.9	.....	2	5	4
2	15	(FT + 5)	55.0	75.0	36.3	55.4	.....	34.45	25.91	30.18 ± 1.38	.....	56.1	48.5	52.3	.....	3	S. G.	5
3	15	(FT + 10)	57.5	40.0	41.3	46.3	.....	35.30	24.81	30.06 ± 1.36	.....	56.8	51.8	54.3	.....	2	5	4
4	15	(FT + 15)	60.0	77.5	48.8	62.1	.....	29.38	26.45	27.92 ± 1.28	.....	54.9	52.0	53.5	.....	3	5	4
5	30	FT	52.5	75.0	37.5	55.0	.....	32.72	26.36	29.54 ± 1.35	.....	56.0	51.8	53.9	.....	3	5	4
6	30	(FT + 15)	57.5	70.0	42.5	56.7	.....	28.40	27.07	27.74 ± 1.27	.....	55.1	52.8	54.0	.....	3	4	4
7	30	(FT + 10)	50.0	43.8	37.5	43.8	.....	33.65	24.67	29.16 ± 1.34	.....	57.3	52.8	55.0	.....	2	4	3
8	30	(FT + 15)	50.0	78.8	41.3	56.7	.....	27.65	25.52	26.59 ± 1.22	.....	53.3	50.3	51.8	.....	4	5	5
9	0	Check	76.3	85.0	52.5	71.3	18.36	31.20	24.63	24.73 ± 0.87	46.8	53.5	50.0	50.1	S. G.[c]	4	5	5
10	45	FT	52.5	85.0	33.8	57.1	.....	32.45	26.52	29.49 ± 1.35	.....	55.1	52.8	54.0	.....	3	4	4
11	45	(FT + 5)	47.5	80.0	43.8	57.1	.....	32.85	23.96	28.41 ± 1.30	.....	55.4	49.5	52.5	.....	3	5	4
12	45	(FT + 10)	60.0	57.5	30.0	49.2	.....	33.52	22.45	27.99 ± 1.28	.....	55.9	51.0	53.5	.....	3	5	4
13	45	(FT + 15)	57.5	68.8	40.0	55.4	.....	30.98	21.38	26.18 ± 1.20	.....	54.5	50.0	52.5	.....	3	5	4
14	60	FT	50.0	76.3	25.0	50.4	26.98	33.34	26.85	29.06 ± 1.03	53.7	55.6	51.3	53.5	4	3	5	4
15	60	(FT + 5)	51.3	76.3	30.0	52.5	29.47	38.10	21.03	29.53 ± 1.04	52.6	57.1	49.5	53.1	4	2	5	4
16	60	(FT + 10)	52.5	42.5	27.5	40.8	26.67	28.14	22.18	25.66 ± 0.91	51.1	57.5	50.3	52.6	5	1	5	4
17	60	(FT + 15)	50.0	70.0	33.8	51.3	24.40	30.22	24.05	26.22 ± 0.93	54.3	56.4	49.8	53.5	4	3	5	4
18	0	Check	75.0	77.5	46.3	66.3	18.49	25.51	20.00	21.33 ± 0.75	45.9	54.8	48.5	49.7	S. G.	3	S. G.	5

[a] FT = One application at flowering time.
(FT + 5) = One application 5 days after flowering time.
(FT + 10) = " " 10 " " " " "
(FT + 15) = " " 15 " " " " "

[b] Average of four systematically distributed plots at each locality.

[c] S. G. = Sample Grade.

ences in the mean average percentage of rust on the plots dusted once on the different schedules and at the different rates were not large enough to permit any definite conclusions except that the rust percentage was uniformly the lowest on the plots where the Kolo dust was applied 10 days after flowering time. This, as already pointed out, may have been due to the fact that at Crookston all the plots dusted 8 to 10 days after flowering time were freer from rust than the other plots; consequently, this would lower the mean average percentage of rust for these particular plots.

The mean average yields of wheat from the near-by nondusted plots were 24.72 ± 0.87 and 21.33 ± 0.75 bushels per acre, respectively (Table 3, Plots 9 and 18). These yields compare favorably with 23.31 bushels per acre for the average yield of all the nondusted plots in the entire experiment. We will compare only the yield data for the plots dusted once at the 60-pound rate, since only these particular plots were harvested at Morris. In general, at the three stations, plots dusted once, especially at flowering time, outyielded the nondusted plots. The result would hardly be expected from one application of Kolo dust, since there appeared to be no significant observable evidences of the practical control of rust at the time the rust notes were taken shortly before harvest. This may be a coincidence, but more probably it may have been due to the fact that one application of Kolo dust delayed the development of the rust for a time, although the final percentage was not significantly reduced in comparison with that in the nondusted plots. The mean average yields for the plots dusted once at the 60-pound rate at the flowering time, 5, 10, and 15 days after flowering time showed increases of 5.75, 6.22, 2.35, and 2.91 bushels per acre, respectively, of which only the yields for the plots dusted once at flowering time or 5 days after flowering time are probably significant. It would not be justifiable to draw any conclusions regarding the efficiency of one application at the 15-, 30-, 45-, and 60-pound rates because only the plots dusted at the 60-pound rate were harvested at Morris. However, if the data at Crookston and St. Paul are considered separately or together, there appear to be no significant differences in yield for the four rates of application. The time of application is a far more important factor.

The mean average weights per bushel and the grade of wheat at the three stations were uniformly higher for the plots dusted once than for the nondusted plots. The mean average weights per bushel for the near-by nondusted plots were 50.1 and 49.7 pounds, respectively (Table 3, Plots 9 and 18), and the mean average grade of each was No. 5. These compare favorably with 50.2 pounds per bushel and a grade of No. 5 for the average of 120 nondusted plots in the entire experiment. As in discussing the mean average yields, only the plots dusted once at the 60-pound rate will be considered in a comparison of the mean average weight per bushel and the grade. In each case the mean average grade was No. 4. The weights when

TABLE 4.—*Comparison of mean-average data upon the efficiency of 3, 2, and 1 applications of Kolo dust when applied at rates of 15-, 30-, 45-, and 60-pounds per acre and at various intervals in relation to flowering time of wheat, as measured by the control of stem rust and increase in yield, weight per bushel, and grade of Marquis wheat at Morris, Crookston, and St. Paul, Minnesota, in 1927*

Plot No.	Application			Stem Rust			Yield			Weight per bushel			Grade	
	Rate	No.	Time[a]	Mean Av.[b]	Decrease over check in		Mean average[b]	Increase over check in		Mean average[b]	Increase over check in		Mean average[b]	Increase over check in grades
					Actual	Proportional		Bu.	Per ct.		Lbs.	Per ct.		
56	15	3	FT + 6 + 4	26.1	39.5	60.2	31.62 ± 1.12	8.31	35.6	55.3	5.1	10.2	3	2
20	15	2	FT + 6	37.9	27.7	42.2	30.09 ± 1.06	6.78	29.1	55.2	5.0	10.0	3	2
1	15	1	FT	55.9	9.7	15.0								
57	15	3	FT + 8 + 4	23.6	42.0	64.0	31.47 ± 1.11	8.16	35.0	56.0	5.8	11.6	3	2
21	15	2	FT + 8	40.0	25.6	39.0	30.31 ± 1.07	7.00	30.0	55.5	5.3	10.6	3	2
2	15	1	(FT + 5)	55.4	10.2	15.5								
63	30	3	FT + 6 + 4	21.9	43.7	66.6	33.04 ± 1.17	9.73	41.7	56.1	5.9	11.8	3	2
24	30	2	FT + 6	38.4	27.2	41.5	31.41 ± 1.11	8.10	34.8	55.6	5.3	10.6	3	2
5	30	1	FT	55.0	10.6	16.2								
64	30	3	FT + 8 + 4	17.6	48.0	73.2	34.35 ± 1.21	11.04	47.4	56.2	6.0	12.0	3	2
25	30	2	FT + 8	38.4	27.2	41.5	28.73 ± 1.01	5.42	23.3	55.6	5.4	10.8	3	2
6	30	1	(FT + 5)	56.7	8.9	13.6								
Check	0	0		65.6			23.31 ± 0.82			50.2			5	
70	45	3	FT + 6 + 4	18.8	46.8	71.3	33.68 ± 1.19	10.37	44.5	56.2	6.0	12.0	3	2
29	45	2	FT + 6	52.1	13.5	20.6	31.53 ± 1.11	8.22	35.3	55.6	5.4	10.8	3	2
10	45	1	FT	57.1	8.5	13.0								
71	45	3	FT +8 + 4	15.4	50.2	76.5	30.45 ± 1.08	7.14	30.6	55.8	5.6	11.2	3	2
30	45	2	FT + 8	38.4	27.2	41.5	32.57 ± 1.15	9.26	39.7	55.0	4.8	9.6	3	2
11	45	1	(FT + 5)	57.1	8.5	13.0								
77	60	3	FT+6 + 4	14.0	51.6	78.7	36.27 ± 1.28	12.96	55.6	57.1	6.9	13.7	2	3
33	60	2	FT +6	38.4	27.2	41.5	29.61 ± 1.05	6.30	27.0	54.3	4.1	8.2	4	1
14	60	1	FT	50.4	15.2	23.2	29.06 ± 1.03	5.75	24.7	53.5	3.2	6.4	4	1
78	60	3	FT + 8 + 4	13.0	52.6	80.2	34.18 ± 1.21	10.87	46.6	57.1	6.9	13.7	2	3
34	60	2	FT + 8	32.6	33.0	50.3	30.21 ± 1.07	6.90	29.6	55.0	4.8	9.6	3	2
15	60	1	(FT + 5)	52.5	13.1	20.0	29.53 ± 1.04	6.22	26.7	53.1	2.9	5.8	4	1

[a] FT = One application at flowering time.
(FT + 5) = One application 5 days after flowering time.
FT + 6 = First application at flowering time and the second 6 days after the first.
FT + 8 = " " " " " " " " " 8 " " " "
FT + 6 + 4 = First application at flowering time and the second 6 days after the first, with a third 4 days after the second.
FT + 8 + 4 = " " " " " " " " 8 " " " " " " " " 4 " " " "

[b] Mean average = Data from 12 plots, 4 systematically distributed plots at Morris, Crookston, and St. Paul.

compared with 50.2 pounds, the average of all the nondusted plots, represent increases of 3.3, 2.9, 2.4, and 3.3 pounds, respectively, as well as an increase in grade from No. 5 to No. 4. From the data at Crookston and St. Paul, whether considered separately or together, it is evident not only that the weight and grade of wheat for the plots dusted once are higher than those for the nondusted plots but also that the 15- and 30-pound rates were just as effective as the 45- and 60-pound rates.

A COMPARISON OF THE MEAN AVERAGE DATA OF THE RELATIVE EFFECTIVENESS OF THREE, TWO, AND ONE APPLICATION OF KOLO DUST APPLIED AT DIFFERENT RATES TO MARQUIS WHEAT

When comparing the data for the nondusted plots, the mean average proportional reductions in the amount of stem rust for plots dusted three times at the 15-, 30-, 45-, and 60-pound rates on the F.T. + 6 + 4 schedule were 60.2, 66.6, 71.3, and 78.7 per cent, respectively (Table 4, Plots 56, 63, 70, and 77); and for the F.T. + 8 + 4 schedule, they were 64.0, 73.2, 76.5, and 80.2 per cent, respectively (Table 4, Plots 57, 64, 71, and 78). For the plots dusted twice at the four rates on the F.T. + 6 schedule, the reductions were 42.2, 41.5, 20.6, and 41.5 per cent, respectively (Table 4, Plots 20, 24, 29, and 33); and for the F.T. + 8 schedule, they were 39.0, 41.5, 41.5, and 50.3 per cent, respectively (Table 4, Plots 21, 25, 30, and 34). For the plots dusted once at the four rates on the F.T. schedule, the reductions were 15.0, 16.2, 13.0, and 23.2 per cent, respectively (Table 4, Plots 1, 5, 10, and 14); and for the F.T. + 5 schedule, they were 15.5, 13.6, 13.0, and 20.0 per cent, respectively (Plots 2, 6, 11, and 15). From these data it is evident that two applications of Kolo dust were almost as effective as three and that the 15- and 30-pound rates were almost as effective as the 45- and 60-pound rates.

In comparing the data for the nondusted plots, the mean average increases in the yield of wheat for the plots dusted three times at the 15-, 30-, 45-, and 60-pound rates on the F.T. + 6 + 4 schedule were 35.6, 41.7, 44.5, and 55.6 per cent, respectively (Table 4, Plots 56, 63, 70, and 77); and for the F.T. + 8 + 4 schedule, they were 35.0, 47.4, 30.6, and 46.6 per cent, respectively (Table 4, Plots 57, 64, 71, and 78). For the plots dusted twice at the four rates on the F.T. + 6 schedule, the increases in yield were 29.1, 34.8, 35.3, and 27.0 per cent, respectively (Table 4, Plots 20, 24, 29, and 33); and for the F.T. + 8 schedule, they were 30.0, 23.3, 39.7, and 29.6 per cent, respectively (Table 4, Plots 21, 25, 30, and 34). Comparative data for plots dusted once at the 60-pound rate show increases of 24.7 and 26.7 per cent, respectively (Table 4, Plots 14 and 15). From these data it is evident that two applications were almost as effective as three and that the 15- and 30-pound rates were nearly as effective as the 45- and 60-pound rates.

b Mean average = Data from 12 plots, 4 systematically distributed plots at Morris, Crookston, and St. Paul.

When comparing the data for the nondusted plots, the mean average increases in weight per bushel for plots dusted three times at the 15-, 30-, 45-, and 60-pound rates on the F.T. + 6 + 4 schedule were 10.2, 11.8, 12.0, and 13.7 per cent, respectively (Table 4, Plots 56, 63, 70, and 77); and for the F.T. + 8 + 4 schedule, they were 11.6, 12.0, 11.2, and 13.7 per cent, respectively (Table 4, Plots 57, 64, 71, and 78). For the plots dusted twice at the four rates on the F.T. + 6 schedule, the increases in weight per bushel were 10.0, 10.6, 10.8, and 8.2 per cent, respectively (Table 4, Plots 20, 24, 29, and 33); and for the F.T. + 8 schedule they were 10.6, 10.8, 9.6, and 9.6 per cent, respectively (Table 4, Plots 21, 25, 30, and 34). Comparative data for plots dusted once at the 60-pound rate show increases of 6.4, and 5.8 per cent, respectively (Table 4, Plots 14 and 15). From these data it is evident that two applications of Kolo dust were almost as effective as three and, further, that the 15- and 30-pound rates were nearly as effective as the 45- and 60-pound rates.

Comparing the mean average data for the nondusted plots, the mean average increases in number of grade of the wheat from the plots dusted three times at the 15-, 30-, 45-, and 60-pound rates on the F.T. + 6 + 4 schedule were 2, 2, 2, and 3 grades, respectively (Table 4, Plots 56, 63, 70, and 77); and for the F.T. + 8 + 4 schedule, they were the same, being 2, 2, 2, and 3, respectively (Table 4, Plots 57, 64, 71, and 78). The increases in number of grade of wheat for plots dusted twice at the four rates for the F.T. + 6 schedule were 2, 2, 2, and 1 grades, respectively (Table 4, Plots 20, 24, 29, and 33); and for the F.T. + 8 schedule they were 2, 2, 2, and 2 grades, respectively (Table 4, Plots 21, 25, 30, and 34). Comparative data for the plots dusted once at the 60-pound rate show an increase of 1 grade each (Table 4, Plots 14 and 15). From these data it is evident that, although the increase in number of grade is not so great, two applications were nearly as effective as three and, further, that the 15- and 30-pound rates were practically as effective as the 45- and 60-pound rates.

THE EFFECT OF APPLYING KOLO DUST AT 30-, 45-, AND 60-POUND RATES, AT INTERVALS OF 3, 5, 7, 9, AND 11 DAYS, ON THE CONTROL OF STEM RUST, INCREASE IN YIELD, WEIGHT PER BUSHEL, AND GRADE OF MARQUIS WHEAT

Kolo dust was applied at the 30-, 45-, and 60-pound rates. Dusting was started at flowering time and subsequent applications were made at 3-, 5-, 7-, 9-, and 11-day intervals. The schedule and number of applications are shown on the next page.

The percentage of stem rust was lower on the dusted than on the nondusted plots, especially on those plots where the large number of applications were made at the shorter intervals (Table 5). The average per-

	Number of applications	*Intervals in days*
1.	8	3
2.	5	5
3.	4	7
4.	3	9
5.	3	11

centages of rust on the near-by nondusted plots were 67.5, 65.0, and 71.3, respectively (Table 5, Plots C–88, C–94, and C–100). The average percentages on the plots dusted at the 30-, 45-, and 60-pound rates at 11-day intervals (Table 5, Plots C–93, C–99, and C–105) were 24.5, 28.8, and 13.8, respectively, whereas on the plots dusted at the same rates at the 3-, 5-, 7-, and 9-day intervals, the rust averaged 10 per cent less. From these data it is evident that the 9-day interval is the upper limit for length of time between applications. The 30-pound rate appeared to be just as effective as the 45- and 60-pound rates in the control of rust.

The yields on some of the plots were quite valueless because of the unevenness of the stand of wheat in the portion of the field where this experiment was performed. Quack grass (*Agropyron repens*) was the principal cause of the unevenness. For this reason part of series C and D is omitted. The average yields were higher on the dusted than on the nondusted plots, especially where the large number of applications were made at the shorter intervals. The average yields of near-by nondusted plots were 32.40 ± 2.31, 33.25 ± 2.37, and 28.27 ± 2.01 bushels per acre, respectively (Table 5, Plots C–88, C–94, and C–100). The average yields on the plots dusted at the 30-, 45-, and 60-pound rates at the 11-day interval were 41.25 ± 2.94, 29.34 ± 2.09, and 39.87 ± 2.84 bushels per acre, respectively (Table 5, Plots C–93, C–99, and C–105); for the 9-day intervals, they were 48.76 ± 3.47, 33.61 ± 2.39, and 40.27 ± 2.87 bushels per acre, respectively (Table 5, Plots C–92, C–98, and C–104); at 7-day intervals, they were 48.14 ± 3.43, 44.33 ± 3.16, and 40.27 ± 2.87 bushels per acre, respectively (Table 5, Plots C–91, C–97, and C–103); at 5-day intervals, they were 46.10 ± 3.28, 48.24 ± 3.44, and 40.90 ± 2.91 bushels per acre, respectively (Table 5, Plots C–90, C–96, and C–102); and at 3-day intervals, the yields were still higher, being 48.72 ± 3.47, 49.61 ± 3.53, and 44.47 ± 3.17 bushels per acre, respectively (Table 5, Plots C–89, C–95, and C–101). From these data it is evident that the upper limit for time elapsing between applications is 9 days. It would appear that dusting at 3- or 5-day intervals, which would necessitate 8 and 5 applications, is not very practical, but that 4 applications at 9-day intervals, or possibly 3 applications at 8-day intervals starting at flowering time, would be just as effective, besides being far more practical.

TABLE 5.—*The effect of applications of Kolo dust when applied at the 30-, 45-, and 60-pound rates at intervals of 3, 5, 7, 9, and 11 days, on the control of stem rust, and on yield, weight per bushel, and grade of Marquis wheat at Crookston, Minnesota, in 1927*

Plot No.	Application			Stem rust per cent on the peduncle					Yield per acre in bushels					Weight per bushel in pounds					Average grade No.
	Rate in pounds per acre	No.	Time[a]	Series				Av.	Series				Average	Series				Av.	
				A	B	C	D		A	B	C	D		A	B	C	D		
C– 88	0	0	Check	80	45	80	65	67.5	32.54	32.72	31.83	.....	32.40 ± 2.31	55.0	57.5	55.0	.....	55.8	3
C– 89	30	8	FT + 3 day intervals	tr	3	5	3	2.8	59.92	35.56	50.67	.....	48.72 ± 3.47	55.0	58.0	57.0	.....	56.7	2
C– 90	30	5	FT + 5 " "	tr	2	8	tr	2.5	65.61	38.23	46.58	33.96	46.10 ± 3.28	56.0	53.0	57.0	56.0	56.5	2
C– 91	30	4	FT + 7 " "	30	2	8	tr	10.0	48.18	42.85	49.25	52.27	48.14 ± 3.43	55.0	57.5	56.0	57.0	56.4	3
C– 92	30	3	FT + 9 " "	8	5	15	10	9.5	57.25	48.90	45.34	43.56	48.76 ± 3.47	56.0	57.5	55.0	57.0	56.4	3
C– 93	30	3	FT + 11 " "	18	30	25	25	24.5	44.81	44.09	35.56	40.54	41.25 ± 2.94	56.5	55.0	57.5	56.0	56.3	3
C– 94	0	0	Check	75	60	50	75	65.0	34.14	30.76	.....	34.85	33.25 ± 2.37	53.0	52.5	.....	54.0	53.2	4
C– 95	45	8	FT + 3 " "	10	tr	3	tr	3.3	46.23	62.76	40.89	48.54	49.61 ± 3.53	56.0	56.5	57.0	55.5	56.3	3
C– 96	45	5	FT + 5 " "	3	tr	3	2	2.0	49.78	40.72	.....	54.23	48.24 ± 3.44	54.0	56.5	.....	55.5	55.3	3
C– 97	45	4	FT + 7 " "	25	tr	3	2	7.5	42.32	21.34	.....	49.96	44.33 ± 3.16	55.0	56.5	.....	56.0	55.8	3
C– 98	45	3	FT + 9 " "	15	5	5	15	10.0	39.83	20.27	.....	40.72	33.61 ± 2.39	55.0	54.5	.....	55.0	54.8	3
C– 99	45	3	FT + 11 " "	45	10	45	15	28.8	29.16	19.56	34.14	34.49	29.34 ± 2.09	52.0	55.5	57.0	55.0	54.9	3
C–100	0	0	Check	85	30	85	85	71.3	27.91	23.65	30.40	31.12	28.27 ± 2.01	51.0	56.0	54.0	53.5	53.6	4
C–101	60	8	FT + 3 " "	tr	tr	2	tr	0.5	56.90	33.43	49.25	38.40	44.47 ± 3.17	58.0	58.0	56.0	58.0	57.5	1
C–102	60	5	FT + 5 " "	tr	5	2	2	2.3	32.18	34.14	56.90	40.36	40.90 ± 2.91	56.0	59.0	56.0	55.5	56.6	2
C–103	60	4	FT + 7 " "	5	5	3	3	4.0	42.85	38.05	43.56	36.63	40.27 ± 2.87	57.0	57.0	57.0	60.0	57.8	1
C–104	60	3	FT + 9 " "	10	5	10	tr	6.3	53.34	51.03	32.54	22.58	39.87 ± 2.84	58.0	59.0	57.0	58.0	58.0	1
C–105	60	3	FT + 11 " "	15	10	20	10	13.8	40.36	35.38	40.01	34.67	37.61 ± 2.68	54.5	59.0	57.0	59.0	57.4	2

[a] = FT + 3 day intervals = First application at blossom time with 7 subsequent applications at 3-day intervals.
FT + 5 " " " " " " " " " " 4 " " " " 5- " "
FT + 7 " " " " " " " " " " 3 " " " " 7- " "
FT + 9 " " " " " " " " " " 2 " " " " 9- " "
FT + 11 " " " " " " " " " " 2 " " " " 11- " "

Similar correlations of the control of stem rust and increase in yield with quality of wheat as measured by increases in weight per bushel and grade also were found. As might be expected, the wheat from the plots dusted at the shorter intervals, which necessitated a larger number of applications, weighed and graded higher than that from plots dusted at the longer intervals. As a rule the weight per bushel and grade were higher for the wheat from the dusted than that from the nondusted plots. The wheat from the near-by nondusted plots weighed 55.8, 53.2, and 53.6 pounds per bushel and graded No. 3, No. 4, and No. 4, respectively (Table 5, Plots C–88, C–94, and C–100). The wheat from the plots dusted at the 30-, 45-, and 60-pound rates at 3-day intervals weighed 56.7, 56.3, and 57.5 pounds per bushel and graded No. 2, No. 3, and No. 1, respectively (Table 5, Plots C–89, C–95, and C–101); for the 5-day intervals (Table 5, Plots C–90, C–96, and C–102), they were 56.5, 55.3, and 56.6 pounds per bushel and graded No. 2, No. 3, and No. 2, respectively; for the 7-day intervals (Table 5, Plots C–91, C–97, and C–103), they were 56.4, 55.8, and 57.8 pounds per bushel and graded No. 3, No. 3, and No. 1, respectively; for the 9-day intervals (Table 5, Plots C–92, C–98, and C–104), they were 56.4, 54.8, and 58.0 pounds per bushel and graded No. 3, No. 3, and No. 1, respectively; and for the plots dusted at the 11-day intervals (Table 5, Plots C–93, C–99, and C–105), they were 56.3, 54.9, and 57.4 pounds per bushel and graded No. 3 in the first two instances and No. 2 in the last. From these data it is evident that the wheat from the plots dusted at the 60-pound rate weighed and graded uniformly higher than that for the 30- and 45-pound rates.

Somewhat similar results also were obtained at Crookston when lodged Mindum wheat was dusted on the same schedule and at the same rates, although the control of stem rust was not so evident.

THE EFFECT OF NINE APPLICATIONS OF KOLO DUST WHEN APPLIED AT THE 60-POUND RATE BEFORE, DURING AND SUBSEQUENT TO FLOWERING TIME ON THE SETTING OF SEED, CONTROL OF STEM RUST, INCREASE IN YIELD, WEIGHT PER BUSHEL, AND GRADE OF MARQUIS WHEAT

Since flowering time was arbitrarily selected as the time to start dusting operations on wheat in Minnesota for the control of stem rust, it was thought that Kolo dust might inhibit or be injurious to seed setting, although such an effect was seriously doubted, because wheat is self-fertilized and therefore the possibility of Kolo dust gaining entrance to the florets prior to or at the time of anthesis would be very small. Consequently, Marquis wheat was dusted at the 60-pound rate daily for 6 days, starting 4 days before flowering time. Following these daily applications the plants were dusted three times at weekly intervals. The data obtained are summarized in table 6.

TABLE 6.—*The effect of 9 applications of Kolo dust when applied at the 60-pound rate before, during, and subsequent to flowering time of the wheat plant on the setting of seed and the control of stem rust, and on yield, weight per bushel, and grade of Marquis wheat at Crookston, Minnesota, in 1927*

Plot No.	Treatment	Average number of fertile flowers per spike	Stem rust percentage on peduncle Series					Yield per acre in bushels Series					Weight per bushel in lbs. Series					Average grade number
			A	B	C	D	Av.	A	B	C	D	Av.	A	B	C	D	Av.	
C– 0	Dusted	25.90	tr	tr	tr	tr	tr	65.61	65.61	63.83	.....	65.02	56.5	56.5	56.0	.....	56.3	3
C–82	Nondusted	22.06	95	70	85	80	82.5	26.14	27.74	40.72	35.20	32.45	52.0	56.0	55.4	54.0	54.25	4

In order to determine if there was any injurious effect to Kolo dust when applied before, during, and subsequent to flowering time, 100 spikes from the dusted and 100 from the nondusted plants were selected at random and the number of fertile florets per spike was recorded. It was found that there was no evidence of the failure of seed to set. In fact, the average number of fertile florets per spike was higher for the dusted than for the nondusted plots, being 25.90 and 22.06, respectively.

The dusted plants were almost completely free from stem rust. Only a trace was found in comparison with 82.5 per cent on the near-by nondusted plots.

The average yield of wheat from the plants dusted 9 times was 65.02 bushels per acre in comparison with 32.45 bushels per acre for that of the near-by nondusted plots. This represents slightly over a 100 per cent increase in yield for the dusted plants.

The average weight per bushel for the wheat from the plants dusted 9 times was 56.33 pounds and graded No. 3 in comparison with an average weight per bushel of 54.25 pounds and a grade of No. 4 for that of the near-by nondusted plots. Undoubtedly greater differences might have been found in the quality of wheat in the dusted and nondusted plots, if the former had been allowed to go to full maturity before being harvested. As it was, these dusted plots, still fairly green, were harvested along with the others which were then ripe.

THE EFFECT OF ONE APPLICATION OF KOLO DUST BY BROADCASTING OVER THE SOIL, ON THE CONTROL OF STEM RUST, INCREASE IN YIELD, WEIGHT PER BUSHEL, AND GRADE OF MARQUIS WHEAT

It was thought that there might be some fertilizer effect of Kolo dust that would not be taken into consideration when interpreting the data. Consequently, an experiment was conducted in which Kolo dust was applied broadcast to the soil between the rows of wheat at flowering time, at the following pound rates per acre: 30, 60, 120, 240, 480, 960, and 1,920. The data are summarized in table 7.

There was no observable evidence of the control of stem rust on the plots to which Kolo dust was applied broadcast to the soil when compared with that on the plots to which no Kolo dust was applied. The average percentage of stem rust on the peduncle of the plants in this experiment ranged from 53.8 to 61.3. If it is assumed that the volatile sulphur fumes in Kolo dust are a toxic agent, then there apparently was an insufficient amount of sulphur volatilized at any one time from Kolo dust on the soil to inhibit the development of the rust fungus.

There appeared to be no fertilizing effect by broadcasting Kolo dust to the soil. The average yields of wheat on the plots to which Kolo dust had been applied broadcast were not significantly different from those on the plots to which no Kolo dust had been applied.

TABLE 7.—*The effect of Kolo dust applied broadcast to the soil on the control of stem rust and on yield, weight per bushel, and grade of Marquis wheat sown late at St. Paul, Minnesota, in 1927*

Plot No.	Rate per acre of application in pounds	Stem rust per cent on the peduncle					Yield per acre in bushels					Average weight per bushel in pounds	Average grade number
		Series				Av.	Series				Average		
		A	B	C	D		A	B	C	D			
UF– 165	30	45	65	60	65	58.8	7.56	5.04	6.84	13.34	8.15 ± 0.81	46.0	Sample grade
UF– 166	60	40	65	60	60	56.3	10.85	10.31	12.80	12.98	11.74 ± 1.17	45.5	" "
UF– 167	120	45	55	65	60	56.3	12.98	5.40	12.45	11.91	10.69 ± 1.07	47.5	" "
UF– 168	240	65	65	60	55	61.3	9.42	6.48	12.09	17.78	11.44 ± 1.14	48.0	" "
UF– 169	0	95	45	55	55	62.5	7.70	11.56	11.20	14.22	11.05 ± 1.10	45.5	" "
UF– 170	480	60	45	60	50	53.8	6.84	7.20	11.20	14.94	10.05 ± 1.00	45.0	" "
UF– 171	960	50	50	60	60	55.0	8.89	10.13	11.73	12.62	10.89 ± 1.09	45.0	" "
UF– 172	1920	60	40	60	60	55.0	2.70	10.31	12.27	13.69	9.74 ± 0.97	47.0	" "
UF– 173	0	55	50	65	65	58.8	5.40	3.60	16.18	8.89	8.52 ± 0.85	46.5	" "

Similarly, the weights per bushel and grades of wheat from the plots to which Kolo dust was applied broadcast did not differ significantly from those of the plots to which no Kolo dust was applied.

A similar experiment was carried out at Crookston where Kolo dust was applied broadcast to the soil, at the same rate and time, between the rows of Mindum wheat. There were no observable evidences of the control of stem rust, nor increases in yield, weight per bushel, or grade of wheat from such treated plots.

THE EFFECT OF SEVEN APPLICATIONS OF KOLO DUST WHEN APPLIED AT THE 60-POUND RATE AT 5-DAY INTERVALS ON THE CONTROL OF ORANGE LEAF AND STEM RUSTS, INCREASE IN YIELD, WEIGHT PER BUSHEL, GRADE, AND PROTEIN CONTENT OF RUBY WHEAT

Ruby wheat was sown in three 1/40-acre plots on April 27. One-half of each plot was dusted with Kolo dust at the 60-pound rate. The first application was made on June 27, one week before flowering time, and 6 subsequent applications were applied at 5-day intervals. Notes on the prevalence of orange leaf rust were taken on August 1 and on stem rust on August 8. Three separate square-yard samples were harvested from both the dusted and nondusted portions of each of the 1/40-acre plots on August 8. The data obtained are summarized in table 8.

Since very little stem rust developed at Crookston on Ruby wheat, which was 10 days earlier than Marquis, there were no significant differences in the amount of stem rust on the peduncles of the dusted and nondusted plants. However, there were significant differences in the amount of orange leaf rust on the dusted and nondusted plants. On the former there was only 26.7 per cent in comparison with 97 per cent for the latter. The leaves withered and dried up on the nondusted plants, whereas the leaves on the dusted plants remained green and functioned for a longer time.

The average yield of wheat from the dusted plants was 28.65 bushels per acre in comparison with 21.00 bushels per acre for the nondusted plants. This represents a 7.65 bushel or a 26.7 per cent increase in yield as a result of the reduction in the amount of orange leaf rust and what little reduction there was in the amount of stem rust.

The average weight per bushel for the wheat from the dusted plants was 61.0 pounds in comparison with 56.7 pounds for that of the nondusted plants. This represents an increase of 4.3 pounds per bushel in weight for the wheat from the dusted plants. The wheat from the dusted plants graded No. 1 in comparison with a grade of No. 2 for that of the nondusted plants. As a further indication of the shrinking of the wheat kernels, 250

TABLE 8.—*The effect of 7 applications of Kolo dust when applied at the rate of 60 pounds per acre at 5-day intervals on the control of orange leaf and stem rusts and on yield, weight per bushel, grade, and protein content of Ruby wheat at Crookston, Minnesota, in 1927*

Plot no.	Treatment	Series			Average
		A	B	C	
	Stem rust per cent on the peduncle				
C–400	Dusted	2.00	1.00	3.00	2.00
C–401	Nondusted	4.00	5.00	4.00	4.30
	Leaf rust per cent				
C–400	Dusted	25.00	30.00	25.00	26.70
C–401	Nondusted	98.00	98.00	95.00	97.00
	Yield per acre in bushels				
C–400	Dusted	31.17	28.80	25.96	28.65
C–401	Nondusted	23.47	19.79	19.74	21.00
	Weight per bushel in pounds				
C–400	Dusted	61.00	61.00	61.00	61.00
C–401	Nondusted	57.00	56.00	57.00	56.70
	Weight per 250 seeds in grams				
C–400	Dusted	2.99	2.96	2.88	2.94
C–401	Nondusted	2.37	2.08	2.23	2.23
	Protein content in per cent				
C–400	Dusted	15.88	15.38	13.13	14.80
C–401	Nondusted	14.80	15.44	15.25	15.16
	Grade number				
C–400	Dusted	1	1	1	1
C–401	Nondusted	2	3	2	2

seeds were picked at random from each lot and weighed. The 250 seeds from the dusted plants weighed 0.718 gram more than seeds from the nondusted plants. Undoubtedly orange leaf rust had some effect on the shrinking of the wheat kernels.

The protein content of the wheat from the nondusted was slightly higher than that from the dusted plants. The average protein-content percentage was 15.16 for the former in comparison with 14.80 for that of the latter. Dusting with Kolo dust to control orange leaf rust and stem rust hardly would be expected to affect the protein content of wheat. The wheat from

the nondusted plants was more shrunken and the relative amounts of carbohydrates and water are probably lower than in the plumper wheat from the dusted plants. Consequently, since the protein content is not affected by the rust, it would be proportionately higher in the wheat from the nondusted plants than in that from the dusted plants.

SUMMARY

1. Experiments on the control of cereal rusts by dusting with Kolo dust were carried out at Morris, Crookston, and St. Paul, Minnesota, in 1927. Varieties of wheat most extensively grown in Minnesota were used in these tests. Marquis and Ruby, common bread wheats, and Mindum, a durum wheat, were grown. There were over 2,000 plots in the entire experiment at these three stations.

2. The mean averages of the data on the Marquis wheat plots at Morris, Crookston, and St. Paul, dusted 3 times with Kolo dust, show as high as an 80.2 per cent reduction in the amount of stem rust; a 57.1 per cent increase in yield; and a 13.7 per cent increase in weight per bushel, as well as an increase in the grade of wheat when compared with that of the nondusted plots. The most effective dusting schedule for three applications of Kolo dust at the three stations from the standpoint of timeliness of application was the F.T. + 6 + 4, or F.T. + 8 + 4 schedule, where the second application was made either 6 or 8 days after the flowering-time application followed by a third application 4 days after the second. The 15- and 30-pound rates were found to be essentially as effective as the 45- and 60-pound rates.

3. The mean averages of the data on the Marquis plots dusted twice with Kolo dust show as high as 50.3 per cent decrease in the amount of stem rust; a 39.7 per cent increase in yield; and a 10.8 per cent increase in weight per bushel, as well as an increase in the grade of wheat when compared with that of the nondusted plots. The most effective schedule for two applications of Kolo dust at the three stations was the F.T. + 6, or F.T. + 8 schedule, where the second application was made either 6 or 8 days after the flowering-time application. Again the 15- and 30-pound rates were found to be nearly as effective as the 45- and 60-pound rates.

4. Although with two applications of Kolo dust the control of stem rust was not so great as with three applications, nor the increase in yield, weight per bushel, and grade so high, yet for practical purposes two applications of Kolo dust were almost as effective as three, and further the 15-pound rate was practically as effective as the 60-pound rate.

5. Although there were no significant observable evidences of the control of stem rust with one application of Kolo dust, such treated plots repeatedly yielded slightly more wheat which weighed and graded slightly higher than that for the nondusted plots.

6. At Crookston, when Marquis wheat plots were dusted with Kolo dust at the 30-, 45-, and 60-pound rates at 3-, 5-, 7-, 9-, and 11-day intervals starting at flowering time, the upper limit for length of time elapsing between applications of Kolo dust was found to be 9 days. It would appear that dusting at 5- or 3-day intervals, which would necessitate five or eight applications, is not very practical. Four applications at 7-day intervals or three applications at 8- or 9-day intervals starting at flowering time, which were almost as effective as the larger number of applications at shorter intervals, would be far more practical.

7. At Crookston, Marquis wheat plots dusted with Kolo dust at the 60-pound rate before, during, and subsequent to flowering time of the wheat plant did not inhibit or prevent the setting of seed. No injury whatsoever was observed.

8. At St. Paul, when Kolo dust was applied broadcast to the soil between the rows of Marquis at flowering time in amounts ranging from 30 to 1,920 pounds per acre, there were no observable evidences of the control of stem rust, nor increases in yield, weight per bushel, and grade of wheat from such treated plots in comparison with those for the plots to which no Kolo dust was applied. Similar results also were obtained at Crookston where Kolo dust was applied broadcast to the soil at the same rate and time in a field of Mindum wheat.

9. At Crookston, Ruby wheat plants dusted seven times with Kolo dust at the 60-pound rate at 5-day intervals yielded 26.7 per cent more wheat than nondusted plants. This increase in yield, as well as an increase in weight per bushel and grade, was ascribed to the marked decrease in the amount of orange leaf rust, since there was very little stem rust in the Ruby plots. The protein content of wheat from the nondusted plants was found to be slightly higher than that of the dusted plants, being 15.16 and 14.80 per cent, respectively.

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CROSS–INOCULATION EXPERIMENTS WITH ERIGERON YELLOWS AND PEACH ROSETTE

J. A. McCLINTOCK

Reference to "The Plant Disease Reporter"[1, 2, 3, 4, 5, 6, 7, 8] indicates that peach rosette is on the increase and is spreading farther northward. Its reported presence in Kentucky and Illinois within the past three years indicates that this disease has wild-host plants in these new areas and that natural carriers are spreading it to cultivated hosts of commercial importance.

As early as 1891 Smith (11) suspected that rosette-infected wild plums were a natural source of infection for peaches, and later the writer (10) verified Smith's suspicions.

Failure to add to the contributions regarding the natural means of disseminating peach rosette led the writer to consider the suggestion by Valleau[3] that diseased wild Erigeron plants might be associated with the presence of peach rosette.

According to "The Plant Disease Reporter,"[2] peach rosette was reported from Tennessee by Waite, in 1907, by Essary, in 1913 and 1917, and by Hesler, in 1920, but no mention was made of its association with wild hosts in that State. Since 1922 the writer has observed natural infections of rosette in peaches and plums in Maury, Williamson, Madison, and Knox counties, Tenn. Search in the vicinity of diseased trees in each case failed to locate rosetted wild plums from which such infections might have spread.

Erigeron canadensis L. is a common weed in waste lands and abandoned fields throughout Tennessee. Yellows-infected Erigeron plants are not

[1] [Valleau, W. D.] Peach rosette found in Kentucky. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. **11**: 133. 1927. [Mimeographed.]

[2] United States Department of Agriculture, Bureau of Plant Industry. Peach rosette. Plant Dis. Rptr. Sup. 60: 170–171. 1928. [Mimeographed.]

[3] [Valleau, W. D.] Outbreak of peach rosette. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. **12**: 62–63. 1928. [Mimeographed.]

[4] [Anderson, H. W.] A report of peach rosette from Illinois. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. **12**: 90. 1928. [Mimeographed.]

[5] United States Department of Agriculture, Bureau of Plant Industry. Reports on peach yellows, rosette and little peach. Plant. Dis. Rptr. **12**: 103. 1928. [Mimeographed.]

[6] [Waite, M. B.] Peach rosette in South Carolina. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. **12**: 142. 1928. [Mimeographed.]

[7] United States Department of Agriculture, Bureau of Plant Industry. Peach rosette. Plant Dis. Rptr. Sup. 70: 219. 1929. [Mimeographed.]

[8] [Waite, M. B.] Peach rosette occurs in Oklahoma. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. **13**: 52. 1929. [Mimeographed.]

uncommon wherever this weed grows, and a study of these diseased plants in various sections of Tennessee indicates that they have symptoms strikingly similar to rosette of peach.

Experiments by Kunkel (6) have proved that yellows of China asters, *Callistephus chinensis* Nees, can be transmitted to *Erigeron annuus* L. and *E. canadensis* L.; therefore, it is assumed that in using the diseased wild Erigeron plants the writer was working with the aster yellows virus. *Erigeron canadensis* is the only species of Erigeron used in these experiments.

Cross-inoculation experiments with aster yellows and peach yellows conducted by Kunkel (6) led the writer to suspect that yellows in asters and its other host plants was different from the various virus diseases of the peach. However, the suggestion by Valleau[9] brought to attention the fact that peach rosette is distinct from peach yellows and has a greater known host range and might, therefore, be transmitted to Erigeron with symptoms similar to aster yellows and yet appear in peach and plum as a rosette disease.

As the control of these virus diseases is closely associated with a knowledge of their wild as well as their cultivated hosts, it seemed desirable to study the relation between Erigeron yellows and peach rosette.

In *Erigeron canadensis* the causal agent of yellows is virulent, like the causal agent of rosette in peach, often killing the host quickly, as contrasted with mosaics in other annuals and yellows in peaches. This is illustrated

FIG. 1. A. A plant of *Erigeron canadensis* which became naturally infected with aster yellows early in the season. This plant attained a height of 16½ inches and died by August 5, 1930, without producing any seed. B. A peach tree completely infected with rosette. Note the greatly shortened internodal growth. Compare with A and figure 2, A.

[9] *Loc. cit.*, 3.

by figure 1, A, a natural infection of Erigeron which was first observed June 18, 1930, and was entirely dead when observed August 5, 1930. Numerous similar cases were observed during 1929. Reference to figure 1, A, also shows another similarity between Erigeron yellows and peach rosette (Fig. 1, B), namely, the greatly shortened internodal growth, which produces a rosetted appearance in both cases. This Erigeron plant, infected early in the season, attained a height of only $16\frac{1}{2}$ inches. While in contrast numerous healthy plants of *E. canadensis* attained a height of 6 to 8 feet before being killed by cold weather in the late fall of 1929.

In cases where Erigeron plants attain considerable size before being infected, only the new growth shows the typical yellows appearance (Fig. 2, A). The same is true of peaches and plums that become infected with peach rosette after considerable growth has been made during a given growing season. *Erigeron canadensis* may also have the one-sided type of infection described by Kunkel (6) in asters. This is also true of peaches and plums in cases of early rosette infection. Peach trees infected by rosette

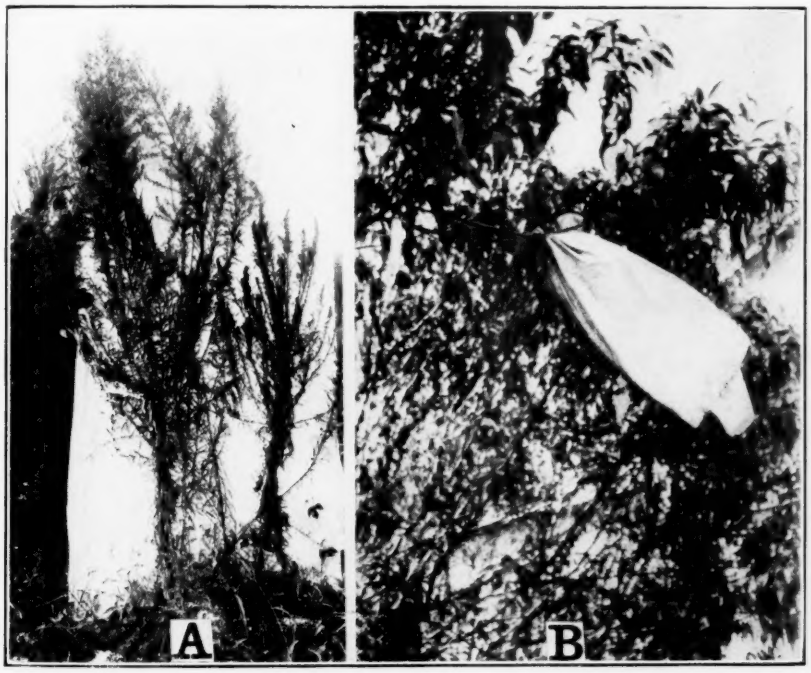

FIG. 2. A. Left, a healthy plant of *Erigeron canadensis*. Right, a similar plant which became infected with aster yellows after it had attained considerable size. Note the shortened internodal development on all of the new top growth of this plant. B. A peach limb with attached cloth bag used as a cage to hold yellows-infected Erigeron plant and its associated insects in close contact with tender peach shoots and leaves.

develop a pale yellowish green color, in contrast to the normal green of healthy peach trees. This is strikingly the case in *E. canadensis* infected with yellows.

Peach trees that become infected with rosette after they have set fruit may mature that crop, yet trees that show a complete rosetted condition early in the spring generally fail to set any fruit, even though they have produced blossoms. Reference to figure 1, A, shows that early natural infection of *Erigeron canadensis* with yellows also results in the failure of the plant to produce seed. Numerous cases of this kind were observed in 1929.

EXPERIMENTS ON SEED TRANSMISSION OF ERIGERON YELLOWS

Reddick and Stewart (10), McClintock (7), Gardner and Kendrick (5), Dickson (2), Archibald (1), and Fajardo (4) have found that the seed of various mosaic-infected leguminous plants transmitted the causal agent; while in the composite plants Kunkel (6) has proved that yellows-infected asters did not transmit the causal agent through the seed. Kunkel states, however, "It is possible that yellows may be transmitted through the seeds of some host plant other than asters." As the work of Doolittle and Walker (3) showed that the seed of the wild cucumber (*Micrampelis lobata* (Michx.) transmits the causal agent of cucumber mosaic, while the seeds of cultivated cucumbers, muskmelons, pumpkin, and squash do not, it seemed desirable to test the seed of *Erigeron canadensis* as one of the wild hosts of aster yellows.

Under the mild weather conditions of Tennessee, *Erigeron canadensis* is often a winter annual. The seeds germinate and produce small plants which grow slowly throughout the fall and winter and complete their growth during the following spring and summer. Thirty-four such plants, dug at random over the University farm and brought to the greenhouse December 13, 1929, developed no symptoms of yellows, and in August, 1930, matured seed the same as seedlings started in flats in the greenhouse.

Plants of *Erigeron canadensis* that become infected with yellows after making considerable growth may mature some seed. During the summer and fall of 1929 mature seed was collected from several typical yellows-infected Erigeron plants growing on the University farm. On November 2, 1929, seed from two of these diseased plants was planted in two flats of good greenhouse soil and held on a greenhouse bench. This seed was somewhat slower in germinating than seed from healthy plants planted in other flats on the same date, but the hundreds of seedlings from the two yellows-infected Erigeron plants grew normally and never developed any symptoms of disease. These plants were not transplanted, but during August, 1930, the few plants left in the greenhouse were producing seed the same as any other healthy plants.

On May 22, 1930, another flat was thickly planted to seed collected from one yellows-infected Erigeron plant on August 6, 1929. In this case the seedlings were above ground in 8 days, the same as those from seed of healthy plants seeded on the same date. Both lots of seedlings were allowed to grow in their respective flats on greenhouse benches until August 14 when they were transplanted to 4-inch pots. None of the 378 plants from seed collected from the yellows-infected Erigeron plants showed any symptoms of yellows during this 84-day period.

These tests indicate that seed from plants of *Erigeron canadensis* infected with yellows do not transmit the causal agent to their seedlings and that Erigeron seeds are probably not a factor in disseminating yellows.

EXPERIMENTS ON INSECT TRANSMISSION FROM YELLOWS-INFECTED ERIGERON TO PEACH

Observations of both healthy and diseased plants of *Erigeron canadensis* in various stages of development disclosed the fact that they are frequented by various insects, including numerous unidentified beetles and grasshoppers; tarnished plant bugs, *Lygus pratensis* Linnaeus; several leaf hoppers, as *Empoasca mali* LeBaron, *Empoasca flavescens* Fab., and *Cicadula sexnotata* Fall; and some aphids, as the green peach aphid, *Rhopalosiphum persicae* Sulzer, the green potato aphid, *Macrosiphum solanifolii* Ashmead; the potato flea beetle, *Epitrix cucumeris* Paris; and the twelve-spotted cucumber beetle, *Diabrotica duodecimpunctata* Olivier. The bunching of the leaves on the yellows-infected Erigeron plants furnished good hiding places for insects, and it was surprising how many could be found on a single diseased plant.

To obtain material for mass insect transfers, the writer used new porous-cloth bags with a draw string at the top and a cloth label sewed in at the bottom. These bags, measuring about 17 inches in length and 7 inches in diameter, when fully opened, were taken to the field to serve as insect cages. By selection among the yellows-infected plants of *Erigeron canadensis*, typical ones were obtained that were a size suitable to enclose within a bag. By drawing an open bag down over the top of a plant, then breaking off the stem and quickly pulling the draw string, it was possible to cage most of the insects on a diseased plant. The insects on 10 typical yellows-infected Erigeron plants were thus caged in separate bags on August 6, 1929. These were carried at once to a row of healthy peach trees, where, one by one, the bags were carefully opened and slipped over the ends of peach limbs. The draw strings were then wrapped several times around the closed mouth of each bag and tied, thus holding the enclosed yellows-infected Erigeron tops and the associated insects in close contact with tender growing peach shoots and leaves, as seen in figure 2, B.

On August 14, or 9 days after the 10 bags were tied about the peach limbs, 5 were loosened and removed. The peach limbs and leaves showed no ill effects from enclosure in the porous-cloth bags, but the Erigeron tops were wilted and dry. Various insects were still alive; therefore, they must have fed on the enclosed peach leaves after the Erigeron tops had dried. The other 5 bags were left closed about the peach shoots throughout the fall, winter, and spring. When these bags were removed, May 1, 1930, the portions of the limbs that had been within the bags were dead or dying; while the rest of each limb appeared normal and fruits had set the same as on other limbs. At this time each of the 10 trees upon which bags of insects had been caged was carefully examined and found to be normal and bearing a good crop of fruit. These 10 trees were under observation throughout the summer and have matured normal crops of fruit. Up to September 1, 1930, none has developed symptoms of peach rosette, or any other symptoms that might be suspected of coming from infection of diseased Erigeron plants.

As the leaf hopper, *Cicadula sexnotata*, which Kunkel (6) has proved to be a carrier of aster yellows to Erigeron and back to asters, was present, along with other insects on the yellows-infected Erigeron plants enclosed in the bags about the peach limbs, opportunity was afforded for the transfer of the causal agent from diseased Erigeron to peach. The fact that no disease appeared on the 10 peach trees indicates that Erigeron yellows is not readily transmitted to peach and probably is a different disease from peach rosette.

EXPERIMENTS ON MECHANICAL TRANSMISSION OF ERIGERON YELLOWS TO PEACH

On August 6, 1929, a number of Erigeron plants in various stages of yellows were pulled up, root and all, and run through a grinder. The crushed mass was then put in a press and 130 cc. of juice extracted and enclosed at once in a tightly corked bottle. By means of a large hypodermic needle with a fine point, tap-water was injected into a number of peach twigs and fruits to serve as checks. With the same needle, undiluted juice extracted from the yellows-infected Erigeron plants was injected into more than 100 growing peach tips and green fruits. These inoculations included 20 peach trees. No disease developed during the remainder of the season of 1929, and in the spring of 1930 these trees blossomed, foliated, and later bore a crop of normal fruit, the same as the check trees. Up to September 1, 1930, none of these trees showed any symptoms of disease.

These experiments indicate that juice from yellows-infected Erigeron plants will not readily produce rosette when artificially injected into actively growing peach shoots and fruits.

Experiments on the transmission of rosette from plum to peach and cherry by buds

To determine the relative effectiveness of Erigeron yellows, and true rosette of Prunus in transmitting rosette, buds were taken from a typical rosetted plum tree on August 14, 1929, and put in 4 trees of cultivated varieties of peaches and 4 sand cherries, *Prunus pumila* L. In all cases bark buds were inserted and tied with $\frac{1}{4}$-inch rubber strips manufactured for nursery purposes. The buds all united with the respective stocks, but no symptoms of rosette appeared during the rest of the 1929 growing season.

On December 7, 1929, 2 of the 4 sand cherries into which rosette plum buds were inserted were dug from the nursery row and set in 2 10-inch

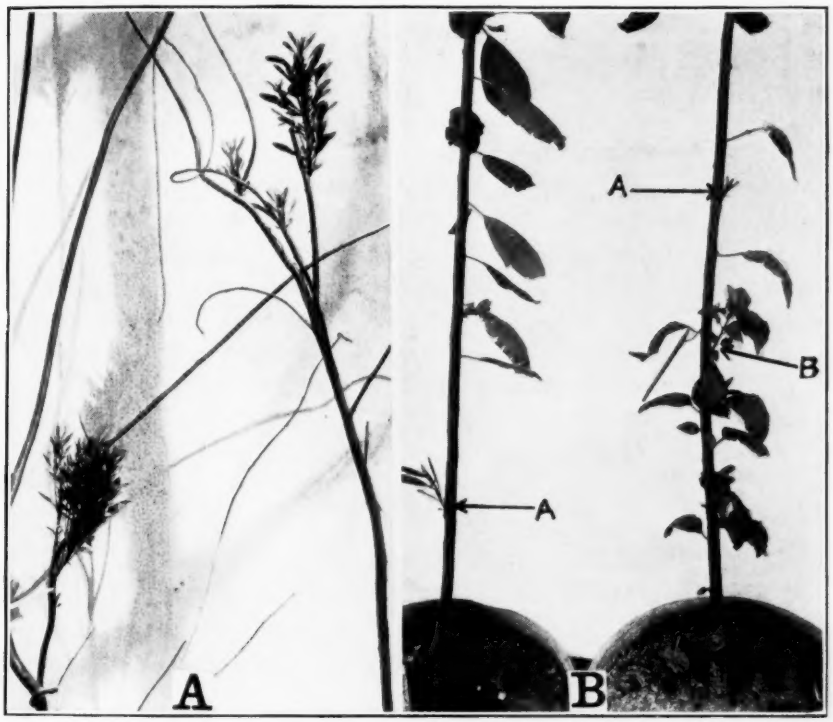

Fig. 3. A. Sand cherries inoculated with buds from a rosette-infected plum. Note the typical rosetted cherry shoots from the few lateral buds which developed. The balance of the lateral, and the terminal buds are still dormant. These 2 sand cherries were the source of inoculum for cross inoculations to Erigeron and plum. B. Plum trees into which dormant buds from rosette sand cherries were inserted April 2, 1930. Note the condition August 26, 1930, with rosette sand-cherry shoots at A and rosette plum shoots at B. Other rosette plum shoots are partly covered by older leaves.

pots, which were transferred to the greenhouse. On February 6, 1930, symptoms of rosette began to appear on the new growth of both sand cherries growing in the greenhouse. The manifestations of rosette on these 2 sand cherries differed from rosette on this host under field conditions in that only a few lateral buds grew into rosetted shoots early in February, while most of the lateral and some of the terminal buds remained dormant, as seen in figure 3, A. Some weeks later additional lateral and terminal buds developed rosetted shoots. In all cases the new growth was typically rosetted, and the few blossoms which opened failed to set fruit. These 2 sand cherries were the source of all rosetted material used in subsequent tests in the greenhouse unless otherwise stated.

With the beginning of new growth in the spring of 1930 the other 2 sand cherries developed typical rosette in all of their new growth. The 4 peach trees budded to rosette plums August 14, 1929, also developed typical rosette in the spring of 1930. Thus rosette was transferred by budding from rosette plum to peach and sand cherry in 100 per cent of the cases, while Erigeron-yellows inoculations made about the same time were entirely negative.

These experiments indicate that conditions were favorable for infection of rosette at the time the Erigeron-yellows inoculations were made in the field during the summer of 1929. This is further evidence that Erigeron yellows and peach rosette are two different diseases.

Incidentally, this is the first record of rosette being artificially transmitted to sand cherries, though one case of natural infection of sand cherries with rosette was observed by the writer during the summer of 1929.

As the pits from which these sand cherries were raised came from large, wild thickets on the sand dunes near Lake Michigan, the presence of rosette in Illinois, as previously mentioned, indicates that this disease is getting near an abundant supply of wild host plants in the North.

EXPERIMENTS ON MECHANICAL TRANSMISSION OF ROSETTE FROM SAND CHERRIES TO ERIGERON CANADENSIS

On February 22, 1930, 4 healthy plants of *Erigeron canadensis* were inoculated with rosette as follows. Leaves were cut from the 2 rosetted sand cherry trees growing in the greenhouse, and the leaf blades and petioles crushed into leaf blades and petioles of the Erigeron plants. The plants were then labeled and at once placed under closed bell-jars. Under these humid conditions the rosetted sand cherry leaves dried very little, but within a few days fungi began to develop on the crushed surfaces, and the bell-jars were therefore removed. The Erigeron plants continued to grow normally on the greenhouse benches. By May 9, 1930, these plants were about 18 inches tall and appeared as normal as check plants which

had had only tap-water crushed into their leaves and petioles on February 22. On August 12, 1930, both the inoculated plants and checks had matured seed without showing any symptoms of disease.

On March 13, 1930, a succulent shoot from a rosette sand cherry was crushed into the tips of each of the new leaves of a healthy potted Erigeron plant and the plant at once placed under a low closed bell jar and left until March 15. The film of moisture that collected on the under side of the bell jar indicated that the air inside of the jar was very humid during this 3-day period. By August 12, 1930, this plant and its check had each matured a crop of seed without either showing any symptoms of disease.

On March 13 exudate from the leaf glands of rosetted sand cherries was transferred on a flamed dissecting needle and pricked into the leaf blades and petioles of a potted Erigeron plant. Tap water pricked into a similar plant with a flamed needle served as a check. The plants were placed under closed bell jars for several days and then held on the greenhouse bench. By May 9 these plants had reached a height of 10 inches and both appeared healthy. By August 12 these plants had matured crops of seed without showing any symptoms of disease. These experiments indicate that the causal agent of rosette cannot be readily transmitted from sand cherries to plants of *Erigeron canadensis* by the ordinary mechanical methods used in transferring virus diseases of the mosaic type in other plants.

On May 17, 1930, two bark buds were cut from 1930 rosette growth of sand cherries and put in T-shape slits through the bark of 2 plants of *Erigeron canadensis* 18 inches tall, growing in 6-inch pots. At the same time 4 wood buds were cut from 1-year-old growth of a plum that had shown typical rosette throughout the season of 1929. These buds were set in T-shape slits through the bark of 4 Erigeron plants 15 to 18 inches tall, growing in 6-inch pots. Two strips of bark without buds were cut from 1-year-old shoots of the same rosetted plum and put into slits through the bark of 2 Erigeron plants 16 inches tall, growing in 6-inch pots. All of these buds and bark strips, as well as checks, consisting of healthy buds and bark strips similarly inserted, were tied with ¼-inch rubber strips. There was no expectation that tissue union would take place between the sand cherries or plums and the Erigeron plants, but it was known that this method would hold rosette Prunus tissues in close contact with succulent Erigeron stem tissues. Both the inoculated plants and the checks continued to grow normally on the greenhouse bench and by August 12 were producing flowers and seed without showing any symptoms of disease. These experiments further indicate that rosette of species of Prunus is not readily transmitted to plants of *E. canadensis* by mechanical means. Together

with the other experiments, this failure of mechanical transfer indicates that peach rosette is not the same as Erigeron yellows.

EXPERIMENTS ON INSECT TRANSMISSION OF PEACH ROSETTE FROM SAND CHERRIES TO ERIGERON

As these experiments were conducted in limited greenhouse space, the writer had to rely on such insects as chanced to be available.

In experiments with spinach blight McClintock and Smith (8), and, later, in studies on peach rosette, McClintock (9) observed that feeding aphids that were quickly or roughly removed from plants on which they were feeding were often injured so that they did not thrive when transferred to a new host. If similar feeding aphids were disturbed by being brushed lightly with a small camel's-hair brush they usually ceased feeding and began to crawl about on the host plant. They could then be removed with the brush and, when transferred to a new host, would generally quiet down and resume feeding in a short time. This method was used in all of the following greenhouse transfers of aphids and appeared to be successful except in the case of the melon aphid, as mentioned later.

TRANSMISSION EXPERIMENTS WITH GREEN PEACH APHIDS

On March 13, 1930, a number of young and mature green peach aphids, *Rhopalosiphum persicae* Sulzer, that had been feeding for more than a week on succulent, rosette sand-cherry shoots in the greenhouse, were removed to a half Petri dish with a camel's-hair brush. The aphids were brushed from the dish and allowed to fall onto the leaves of several potted Erigeron plants. After labeling, the pots were held on the greenhouse bench for several days; then the aphids were killed by spraying with a commercial brand of pyrethrum soap. The plants continued to grow normally and, early in August, matured a crop of seed without showing any symptoms of disease.

On March 13, 6 adult green peach aphids which had been feeding for more than a week on rosette sand-cherry shoots were removed with a camel's-hair brush, and 3 were transferred to each of 2 healthy potted Erigeron plants. After labeling, the pots were enclosed in a bell jar for several days. The aphids were then killed with a pyrethrum soap spray. By May 9 the 2 plants had reached a height of 8 and 12 inches, respectively, and showed no symptoms of disease. By August 12 both plants were nearing maturity and appeared as normal as the check plants.

On March 17, an adult green peach aphid, which had fed more than a week on rosette sand cherry tissue, was transferred by a camel's-hair brush and dropped onto a healthy Erigeron plant. During the several days this plant was held under a bell jar, a colony of young aphids became well estab-

lished on it. About two weeks later the plant was freed of all aphids by means of a pyrethrum-soap spray. This plant continued to grow normally and by early in August had produced a crop of seed without showing any symptoms of disease.

On April 8, 1930, two healthy Erigeron plants in 3-inch pots were set on a 10-inch pot in contact with peach seedlings to which 25 green peach aphids had been transferred from rosette sand cherries on April 2. Aphids readily crawled from the peach seedlings to the Erigeron plants, which were then moved to the greenhouse benches and left for 2 weeks, after which the aphids were killed with a pyrethrum-soap spray. By May 9 these 2 Erigeron plants had reached a height of 12 inches and were apparently quite normal. By August 5 these plants had matured seed without showing any symptoms of disease.

In all cases green peach aphids appeared to thrive on the Erigeron plants after being transferred from the rosette sand cherries.

These tests indicate that the green peach aphid did not transmit rosette from sand cherries to plants of *Erigeron canadensis*.

TRANSMISSION EXPERIMENTS WITH MELON APHIDS

On March 16, 20 melon aphids, *Aphis gossypii* Glover, which had been established for some time on the new growth of rosette sand cherries, were transferred with a camel's-hair brush to a half Petri dish and dropped from there onto 2 healthy Erigeron plants growing in separate pots. The pots were at once enclosed in a bell jar. These aphids failed to become established on Erigeron plants. They crawled about over the plants and pots and onto the soil and the inside of a bell jar but did not appear to feed or reproduce as the green peach aphids had done. Within a few days these aphids all disappeared from the Erigeron plants. The 2 plants continued to grow normally and, by early August, had matured seed without showing any symptoms of disease.

TRANSMISSION EXPERIMENTS WITH BLACK PEACH APHIDS

On April 12, 1930, 22 black peach aphids, *Aphis persicae-niger* Smith, in various stages of development, which had been established on succulent rosette sand-cherry shoots for several weeks, were transferred with a camel's-hair brush to a half Petri dish. From this they were dropped onto 3 healthy Erigeron plants growing in separate pots. After being labeled, the pots were enclosed in bell jars for 4 days; then removed and allowed to grow on the greenhouse bench, after which the aphids were killed with a pyrethrum-soap spray. No symptoms of disease had developed on these plants by early August, when they were maturing seed.

TRANSMISSION EXPERIMENTS WITH MEALY BUGS

Shortly after the potted sand cherries began to develop new rosetted shoots in the greenhouse they became infested with the common mealy bug, *Pseudococcus citri* Risso. These insects appeared to thrive on the rosette sand cherries in the greenhouse but failed to survive when taken to the open.

On March 13, with the aid of a dissecting needle and a camel's-hair brush, 2 of these mealy bugs were transferred from the underside of rosette sand-cherry leaves to a healthy Erigeron plant growing in a 3-inch pot. While the mealy bugs appeared to establish themselves at once on the lower surfaces of Erigeron leaves, they were both found dead when the plant was removed from under a bell jar 5 days later. The Erigeron plant continued to thrive on the greenhouse bench and, by August 12, had matured seed without showing any symptoms of disease.

TRANSMISSION EXPERIMENTS WITH TARNISHED PLANT BUGS

On March 13, 2 tarnished plant bugs, *Lygus pratensis* Linnaeus, captured in a commercial peach orchard, were placed in a porous-cloth bag drawn over a clump of succulent rosette sand cherry shoots. After 3 days in this cloth cage the 2 tarnished plant bugs were freed under a bell jar containing a healthy Erigeron plant. While the bugs were considerably disturbed by their transfer, they soon crawled to the Erigeron plant, where they remained for several days. When the bell jar was removed, later, both bugs were found dead. This Erigeron plant continued normal growth on the greenhouse bench and, by early August, had matured seed without developing any symptoms of disease.

These tests indicate that melon aphids, black peach aphids, mealy bugs, and tarnished plant bugs do not readily transmit rosette from diseased sand cherries to plants of *E. canadensis*.

BUD INOCULATIONS FROM ROSETTE SAND CHERRIES TO PLUMS

To determine the ability of the sand cherry to transmit rosette to other susceptible hosts, as well as to learn whether conditions were favorable for rosette transmission at the time the mechanical and insect transmission experiments were under way in the greenhouse, the following experiment was conducted.

As the rosette sand cherries with which the above greenhouse cross-inoculation experiments were conducted developed as infections from rosette plum buds, it seemed desirable to test the ability of the rosette sand cherry buds to produce rosette in plums. For this purpose dormant wood buds were taken from a branch of 1 of the potted rosette sand cherries April 2, 1930, and put in succulent shoots of plum trees which had been dug from the nursery row, set in 10-inch pots, and brought into the greenhouse December 7, 1929. The buds were tied with $\frac{1}{4}$-inch rubber strips, but

no attempt was made to force their growth by breaking over or removing the plum tops above the sand-cherry buds. Several weeks later the rubber strips were removed and the buds were found to be united with the stocks. These plum trees continued apparently normal growth in the greenhouse until June 3, when 4 axillary shoots on 1 plum stock below the inserted rosette sand-cherry bud began to show a yellowish appearance of the leaves suspiciously like rosette. No axillary buds had started growth on this stock above the inserted sand-cherry bud, and no axillary bud growth had developed on the other potted plum in which a rosette sand-cherry bud was inserted on the same date.

As the temperature in the greenhouse was becoming too high for good growth, all potted trees except the 2 rosette sand cherries were transferred June 9 to a cold frame near the greenhouse, where they could be watered.

By August 5, 1930, 7 yellowish axillary shoots had developed below the rosette sand-cherry bud, which also had developed a weak rosetted tuft of leaves on the plum that showed 4 axillary shoots by June 3, from rosette sand-cherry bud inserted April 2. In the other plum the sand-cherry bud had developed a short rosetted shoot, but no buds had started on the plum stock. By August 26, when the photograph for figure 3, B, was taken, both of the sand-cherry shoots showed typical rosette, and the plum shoots also showed additional symptoms of rosette in the sprouting of lateral buds on the older axillary shoots below the inserted sand-cherry bud.

Additional symptoms of rosette will develop in the plums inoculated with rosette sand-cherry buds, but the experiment has gone far enough to prove that the potted sand cherries from which the mechanical and insect transfers were made to *Erigeron canadensis* contained the causal agent of rosette. This experiment also proves that conditions in the greenhouse were favorable for the transfer of the causal agent of rosette at the time the cross inoculations were made.

SUMMARY

Hundreds of plants of *Erigeron canadensis* L., raised from seed collected from yellows-infected plants, have shown no symptoms of yellows; therefore, it is concluded that seeds of this wild host do not transmit the causal agent of aster yellows.

Numerous unidentified beetles, and grasshoppers, as well as the tarnished plant bug, *Lygus pratensis* Linnaeus; the potato flea beetle, *Epitrix cucumeris* Paris; the 12-spotted cucumber beetle, *Diabrotica duodecimpunctata* Olivier; the leaf hoppers, *Empoasca mali* LeBaron, *Empoasca flavescens* Fab., and *Cicadula sexnotata* Fall; and the aphids *Rhopalosiphum persicae* Sulzer and *Macrosiphum solanifolii* Ashmead, when transferred with yellows-infected plants of *Erigeron canadensis* and caged on healthy peach trees, failed to transmit any symptoms of disease to the peaches.

More than one hundred mechanical inoculations into peach shoots and green fruits, made with a hypodermic needle and a fresh undiluted extract from yellows-infected plants of *E. canadensis*, gave entirely negative results.

Transmission of rosette from plum to peach and sand cherry by infected buds was 100 per cent effective.

The results of mechanical transmission experiments with the causal agent of rosette from sand cherries to *E. canadensis* were entirely negative.

Experiments on the transmission of the causal agent of rosette from sand cherries to plants of *E. canadensis* by the green peach aphid, *Rhopalosiphum persicae* Sulzer, the black peach aphid, *Aphis persicae-niger* Smith, the melon aphid, *Aphis gossypii* Glover, the common mealy bug, *Pseudococcus citri* Risso, and the tarnished plant bug, *Lygus pratensis* Linnaeus, all proved negative.

Bud transmission of the causal agent of rosette from sand cherries to plums was 100 per cent effective.

The results of these cross-inoculation experiments indicate that aster yellows of the wild host, *E. canadensis*, is a disease distinct from rosette of peach, plum, and sand cherry.

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FURTHER STUDIES ON THE SEED-CORN MAGGOT AND BACTERIA WITH SPECIAL REFERENCE TO POTATO BLACKLEG[1]

J. G. Leach

In 1925 (5) and 1926 (6) the writer called attention to the rôle played by the seed-corn maggot (*Hylemyia cilicrura* Rond.)[2] in the epiphytology of potato blackleg. Among other things it was pointed out that a number of unidentified species of bacteria were found constantly associated with the insect in all its stages and that some of these were pathogenic and capable of causing blackleg. The bacteria were demonstrated to be present by cultural means only; no histological studies had been made.

It was also shown that sterile maggots would not grow on sterile potato tubers but would grow normally if bacteria were added. This indicated that the bacteria were beneficial to the larvae and under the conditions of the experiment, essential for their normal development.

Since the publication of the above-mentioned papers it has been possible to continue the study of certain phases of the problem. Although the results are by no means complete, what has been learned should be of some aid to a better understanding of the significance of this relationship between insects and bacteria.

THE INTERNAL BACTERIAL FLORA OF THE SEED-CORN MAGGOT COMPARED WITH THAT OF BLACKLEG PLANTS AND WITH CERTAIN SOIL INHABITING BACTERIA

Although pathogenic bacteria were very frequently obtained in culture from the eggs, the larvae, and the pupae, as well as the adult fly, they were never found to occur in pure culture. One or more nonpathogenic species always occurred in association with the pathogenic ones. In some cases only the nonpathogenic species were obtained. Early in the course of the work it was noticed that these nonpathogenic species were apparently similar to the nonpathogenic bacteria frequently found associated with the blackleg pathogene in diseased plants. It was thought desirable to study in some detail these bacteria commonly associated with the insect and to compare them with those associated with the blackleg disease. It

[1] Published with the approval of the Director as Paper No. 964 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Identification of the insects mentioned in this paper, as well as in the previous papers, is based on determinations made by Dr. J. M. Aldrich of the National Museum and Dr. O. A. Johannsen of Cornell University.

also seemed important to isolate and identify the pathogenic species and to determine to what extent they normally were associated with the insect.

Several hundred cultures have been made from internal parts of the larvae, puparia, and adult flies. A considerable number of apparently different species were isolated. Some may have arisen from chance contamination but several were obtained with such frequency that they could be considered as fairly constant inhabitants. Twenty-two of the most frequently occurring species were selected for more careful examination.

Any one who has attempted to obtain a pure culture of the blackleg pathogene from a diseased plant knows that it is sometimes very difficult to separate the pathogene from the nonpathogenic bacteria that are usually closely associated with it. In morphology and cultural characteristics these saprophytes frequently resemble very closely the true pathogene. Five of the cultures most frequently found in association with blackleg were selected for comparison with those obtained from the insect.

In some experiments, in which potato tubers were inoculated with bits of soil or small quantities of soil extract, it was found that a fairly constant group of bacteria were generally associated with the decay that followed. Because the eggs of the seed-corn maggot are commonly deposited in the soil, it was decided to select a number of these soil bacteria for comparison with the other two groups. Several known species of pathogenic, soft-rotting bacteria also were included in the comparison.

At first there was considerable difficulty in obtaining pure cultures of some of the species but, by repeated plating, the different cultures were finally isolated.

These cultures, 43 in all, were studied morphologically and physiologically to determine those characters most commonly used for identifying bacteria.[3]

In these determinations the methods outlined by the Society of American Bacteriologists (1) were followed in general, although in several cases the results were checked by other slightly different methods.

For staining flagella the method described by Gray (2) was used. The method was found relatively simple and quite reliable.

The alkaline gentian-violet Gram stain was employed. Each determination included a gram-positive and a gram-negative organism, one on either end of a slide with the unknown between them.

Pathogenicity was determined by first inoculating carrot slices and potato tuber slices in Petri dishes incubated at 20° C. All cultures that

[3] The major portion of these determinations were made in the laboratory of Professor S. G. Paine, of the Imperial College of Science and Technology. The author wishes to express his gratitude to the Imperial College for the facilities granted and to Professor Paine for helpful suggestions.

produced a decay in these tests were further tested by inoculating potato stems in the greenhouse. Only those that produced a rapid vigorous decay in all 3 tests were considered pathogenic.

The oxygen relations were based on growth in the closed arm of fermentation tubes.

Liquefaction of gelatin was determined by means of a stab culture on plain gelatin, incubating for 2 months at 20° C. followed by a month at room temperature.

The carbohydrate reactions were tested on beef extract to which 1 per cent of the sugar used was added before sterilization.

Litmus indicator and Dunham tubes were used for the detection of acid and gas.

The reduction of nitrates was detected by the sulphanilic acid-α-naphthylamine test.

Diastatic action was determined on 2 per cent soluble-starch beef-extract agar tested with a saturated solution of iodine in 50 per cent alcohol.

The Ehrlich test for Indol was employed, and in all cases where positive results were obtained the reaction was very marked.

The reaction of all differential media was adjusted to the neutral point of brom-thymol-blue.

No determination was based on a single test. Duplicates or triplicates were always used and doubtful reactions were always repeated until conclusive results were obtained.

The principal characters as determined are summarized in table 1. In analyzing the data in this table it should be realized that no atttempt was made to study all the different kinds of bacteria found associated with the insects or the disease. The coccus forms and large, spore-forming bacteria were excluded, although they may possibly be of some significance. Many of the cultures were isolated while searching for the pathogene and this without doubt resulted in a certain amount of selection of those species resembling the blackleg pathogene.

The important facts demonstrated by the detailed study of this group of cultures may be summarized briefly as follows:

1. Bacteria, apparently identical morphologically, physiologically, and parasitically with known cultures of the blackleg pathogene, were frequently isolated from the surface of the eggs, from the inside of puparia, and from the intestinal tract of the imago of the seed-corn maggot, as well as from the soil and from potato plants affected with blackleg. (Cultures No. 151–A–B, 201–2–R, 202–B–I–R, 301–1–R, 305–A–O–R, 4–I, and 407.)

2. Bacteria, which agreed morphologically and physiologically with published descriptions of *Pseudomonas fluorescense* (Flügge) Migula, and

TABLE 1.—*Summary of characteristics of 36 cultures of bacteria obtained from the seed-corn maggot in various stages of development, from blackleg plants, and from the soil. Seven cultures of soft-rotting bacteria of known identity are included for comparison*

Culture No.	Source	Index number and brief characterization[a]			
151–A–O	Surface of egg of seed-corn maggot	5020–52020–0211–2210–211	22	UUU	0
151–A–R	"	5010–32120–0111–2210–211	22	"	0
153–B	"	5010–32020–0111–2210–211	22	"	0
154–B	"	5020–52101–0200–2210–211	22	"	0
155–A–O	"	5020–52001–0200–2210–211	22	"	0
201–A–R–G	Inside of puparia of seed-corn maggot	5020–52020–0101–2210–211	22	"	0
201–A–R–W	"	5020–52020–0101–2210–211	22	"	0
201–A–2	"	5020–52100–0000–2210–111	22	"	0
201–2–R	"	5010–32120–0111–2210–111	22	"	0
201–A–3	"	5020–52001–0200–2210–211	22	"	0
201–B–1	"	5020–52001–0200–2210–211	22	"	0
201–B–R	"	5020–32120–1111–2210–111	22	"	0
202–B–1	Intestinal tract of imago of seed-corn maggot	5010–52000–0000–2210–211	22	"	0
202–B–I–R	"	5010–32120–0111–2210–211	22	"	0
202–B–O	"	5020–52100–0000–2210–111	22	"	0
203–A	"	5020–52020–0100–2210–211	22	"	0
204–A–G	"	5010–52100–0222–2210–111	22	"	0
204–A–W	"	5010–52100–0202–2210–111	22	"	0
205–A–1	"	5020–52101–0200–2210–211	22	"	0
205–A–O	"	5010–52020–0100–2210–211	22	"	0
205–A–R–G	"	5020–52020–0100–2210–211	22	"	0
205–A–R–W	"	5020–52001–0200–2210–211	22	"	0
301–1–R	Soil	5010–32120–0111–2210–211	22	"	0
301–2	"	5010–52020–0000–2210–111	22	"	0
302–1	"	5010–52120–0000–2210–111	22	"	0
302–2	"	5010–52020–0000–2210–211	22	"	0
303–1	"	5020–52100–0000–2210–111	22	"	0
303–2–R	"	5010–52120–1111–2210–111	22	"	0
305–A–1	"	5020–52001–0200–2210–111	22	"	0
305–A–O–R	"	5010–32120–0111–2210–211	22	"	0
306–R	"	5020–32120–1111–2210–111	22	"	1
404–4	Blackleg plants	5010–52120–0101–2210–211	22	"	0
407	"	5010–32120–0111–2210–211	22	"	0
408–A–1	"	5020–52101–0200–2210–211	22	"	0
409–2–R	"	5020–52001–0200–2210–211	22	"	0
4–I	"	5010–32120–0111–2210–211	22	"	0
Bacillus phytophthorus Appel I	Furnished by E. F. Smith	5010–32120–0111–2210–211	22	"	0
B. carotovorus	Furnished by A. B. Massey	5010–32120–0111–2210–211	22	"	0
B. phytophthorus 1985L	Furnished by Lister Institute	5010–32120–0111–2210–211	22	"	0
B. phytophthorus 1975L	"	5010–32120–0111–2210–211	22	"	0
B. phytophthorus 1996L	"	5010–32120–0111–2210–211	22	"	0
B. solanisaprus 385L	"	5010–32020–0111–2210–211	22	"	0
B. aeroideae 1984L	"	5010–32120–0222–2210–211	22	"	0

[a] Numbers correspond to those used in the description chart of the Society of American Bacteriologists.

Ps. non-liquefaciens Eisenberg, were isolated from the surface of eggs, the inside of puparia, and the intestinal tract of the imago of the seed-corn maggot, as well as from the soil, and plants affected with blackleg. (Cultures No. 154–B, 155–A–0, 201–A–3, 201–B–1, 205–A–1, 205–A–R–W, 305–A–1, 408–A–1, 409–2–R.)

3. Several other nonpathogenic species of bacteria were found to occur commonly in association with the seed-corn maggot, in the soil, and in plants affected with blackleg.

4. No consistent morphologic, physiologic, or parasitic difference could be found between any of the cultures of the blackleg pathogene with the exception that the culture of *Bacillus solanisaprus* Harrison did not liquefy gelatin, while all other pathogenic cultures liquefied it readily and consistently.

5. *Bacillus aeroideae* Townsend, although differing from other pathogenic species in its action on the sugars, was strongly pathogenic on potato tubers and stems.

The similarity of the bacteria making up the intestinal flora of the seed-corn maggot to those obtained from the soil and from plants affected with blackleg appears to be significant. It is probable that the kinds of bacteria found in the intestines of the insect depend largely upon the substrate upon which the insect has fed. An insect having developed in a plant affected with blackleg would probably harbor the blackleg pathogene as well as some of the associated saprophytes, while one having developed in decaying organic matter in the soil probably would harbor only those saprophytes commonly predominating in the soil or in decaying organic matter.

HISTOLOGICAL STUDIES

Although bacteria may be isolated from the interior of maggots, puparia, and adult insect, their exact location within the body can be determined only by histological methods. Furthermore, since it has not been possible to propagate the insect in cages, histological studies seem to offer the best means of determining whether the bacteria are of any significance in the metabolism of the adult fly. Maggots, puparia, and adults of both sexes have been studied histologically. The results of the study to date have been unsatisfactory in some respects but some significant observations have been made. These will be recorded in this paper, and it is hoped that future studies will clear up some of the yet obscure points.

Maggots and puparia in various stages of development were collected in the field during May and June. Some adults were caught in the field, while others were reared from maggots in the laboratory. All specimens used for sectioning were killed and fixed in Zenker's Solution and embedded in paraffin. Sections were cut, varying from 5 to 12 microns in thickness.

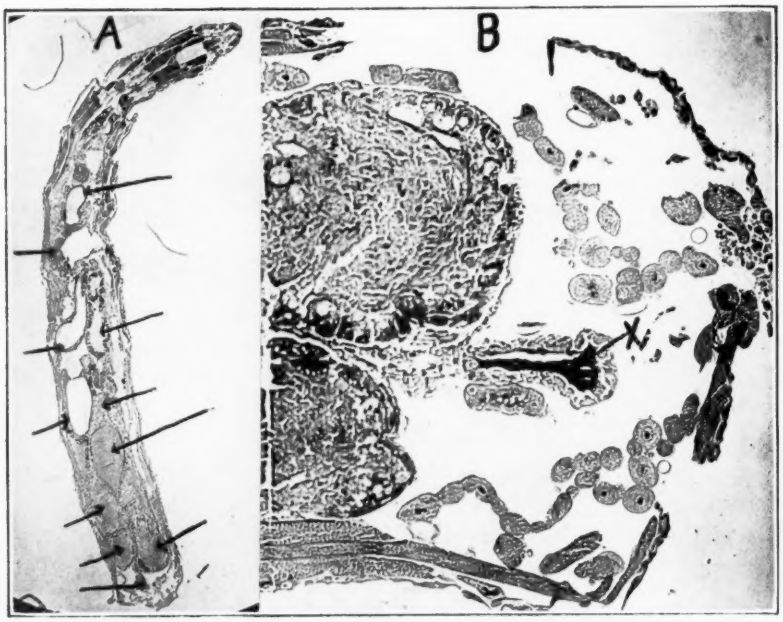

FIG. 1. Photomicrographs showing sections through a larva. A. A longitudinal section; the arrows show the location of intestinal tract containing bacteria mixed indiscriminately with food material. See figure 3 for magnification sufficiently high to show individual bacteria. The bacteria were not concentrated in any special organs. There was no indication that the bacteria were killed or digested by the larva. Approx. 12×. B. Enlarged section of posterior end showing bacteria in anal tract at X. Approx. 100×.

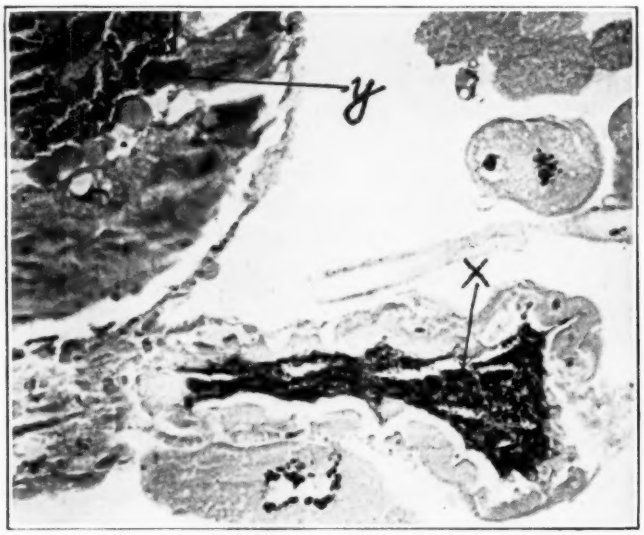

FIG. 2. Same as Fig. 1, B, more highly magnified, showing bacteria at X and Y. Approx. 260×.

Two staining methods were used: that of Goodpasture, as described by Hertig and Wolbach (3); and the Gram-Weigert method, described by Wright and Mallory (8). Aid in interpreting stained microtome sections was obtained by dissecting a large number of freshly killed flies and maggots.

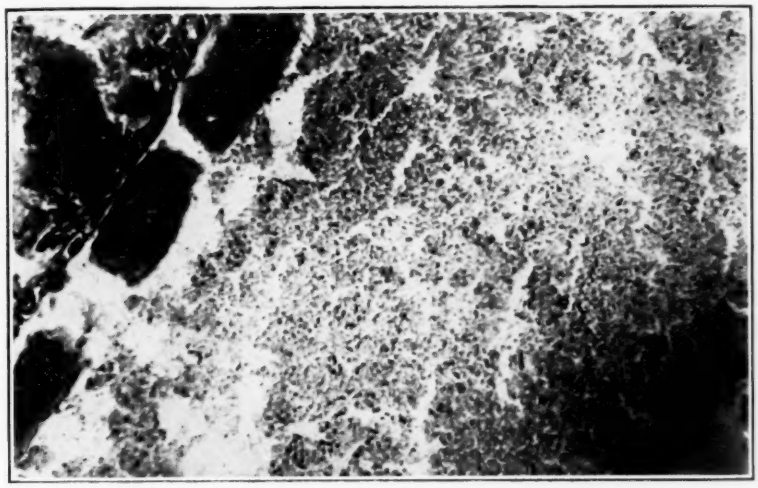

FIG. 3. Section of intestinal contents of a larva, showing short rod-shape bacteria mixed with food material. Approx. 300×. This is from the same larva illustrated in figure 1, A.

The Larva

Although there was a tendency for the larvae to become abnormally lengthened and somewhat discolored during the fixing process, the material yielded fairly good sections which stained well with the Gram-Weigert stain. Bacteria in great abundance were found in the intestinal tract (Figs. 1, 2, and 3). They were in a mixed culture, as indicated by the wide variation in size and shape of the individual cells (Fig. 3). No selective action on the part of the maggot could be detected. The bacteria were mixed indiscriminately with the food material and were not localized in the coecal glands or other special organs. Four long finger-like coecal pouches were found arising immediately behind the proventriculus (Fig. 4), but these did not contain bacteria. Numerous smear stains from freshly dissected material, as well as stained microtome sections, showed them filled with a mixture of spherical and granular bodies embedded in a clear liquid (Fig. 5). However, bacteria were found in the cavity of the proventriculus surrounding the oesophageal valve (Figs. 4 and 6). This cavity may correspond to the "blind sacks" containing bacteria described by Stammer (11), but, if so, it is greatly reduced.

There was no evidence that the bacteria were digested or destroyed by the larva, and they could be found in almost an unbroken mass from one end of the intestinal tract to the other (Figs. 1 and 2). It is quite evident that living bacteria of many different species pass uninjured through the intestinal tracts of the larvae.

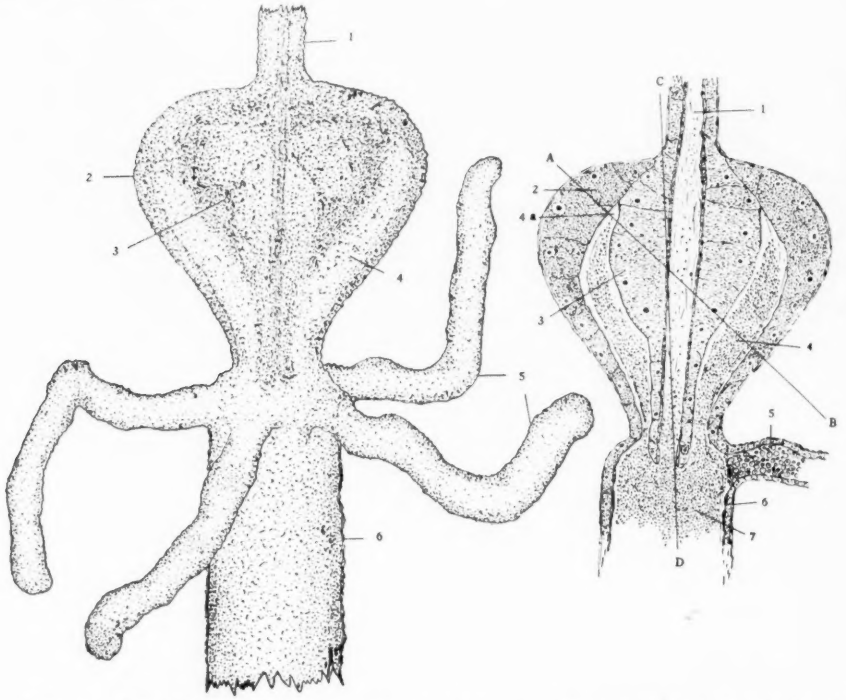

FIG. 4. Semidiagrammatic drawings to show the distribution of bacteria in the proventriculus and anterior portion of the midintestine of a seed-corn-maggot larva. A surface view as seen when dissected under a binocular microscope (at left) and as seen in longitudinal section (at right). 1. Oesophagus. 2. Enlarged portion of midintestine surrounding the oesophageal valve and commonly termed the proventriculus. 3. Oesophageal valve dimly outlined through the wall of the midintestine. 4 and 4a. A space surrounding oesophageal valve containing bacteria and food material. 5. The four coeca filled with spherical bodies of irregular size but containing no bacteria. 6. Midintestine. 7. Bacteria and food materials. For explanation of diagonal lines see figure 6.

The Puparia

The histological studies of the puparia were very unsatisfactory. It was necessary to puncture the puparial case to insure proper fixing; and in certain stages of metamorphosis this apparently resulted in decided disorganization of the enclosed tissues. Sometimes fixation was apparently not complete. In other cases fairly good sections were obtained but the tissues

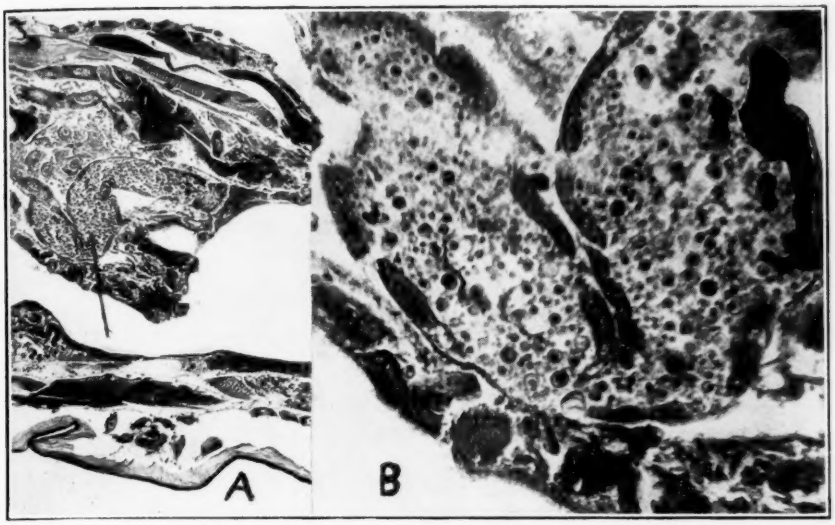

FIG. 5. A photomicrograph of sections through a coecum showing the spherical bodies filling the lumen. A. Magnified approximately 65×. Coecum indicated by arrow. B. Same (approximately 325×), showing the spherical bodies and the absence of bacteria.

FIG. 6. Photomicrographs of sections through the proventriculus, showing bacterial masses surrounding oesophageal valve. A. Cut in plane indicated by line AB. in figure 4. B. Cut in plane indicated by line CD. in figure 4. Numbers correspond to those in figure 4.

did not stain well. In all, approximately 50 different puparia were sectioned and stained. Nearly every possible stage in metamorphosis was included among these but in none of them was it possible to locate bacteria with any degree of certainty. The bacteria apparently lose their affinity for the stain or else become greatly reduced in number. Although the presence of bacteria within the puparia can be demonstrated easily by cultural methods, it has not yet been determined which tissues they inhabit during the metamorphosis of the insect. The solution of this problem must await further work.

The Imago

In order to facilitate fixing the adult insect, the abdomen was usually severed from the thorax. The fixing was satisfactory and the section stained fairly well by both of the staining methods used. Bacteria were rarely found in the thoracic segment but were nearly always present in the abdominal segment in the crop and alimentary canal (Figs. 7 and 8). The number of bacteria present varied with different specimens, apparently depending upon the age of the insect and the amount of food in the intes-

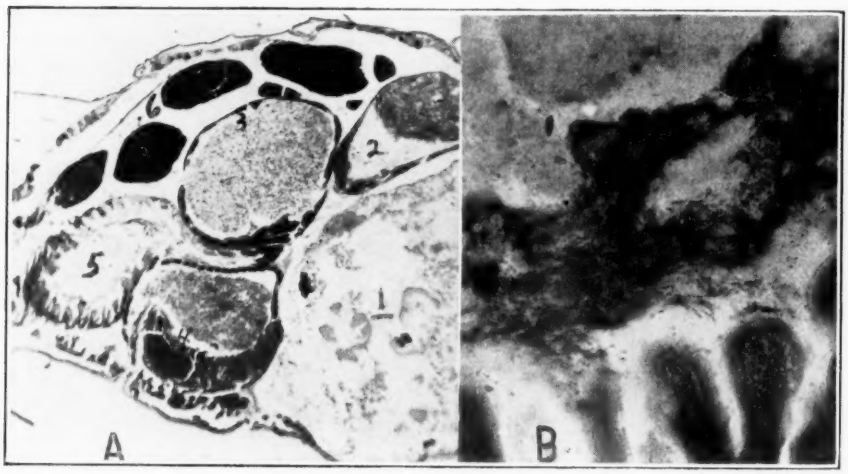

FIG. 7. Photomicrographs showing bacteria in crop and intestinal tract of the imago. A. Dorsoventral longitudinal section of the abdomen of a female imago. 1. The crop containing a liquid matrix in which quantities of bacterial cells are suspended. Higher magnifications are shown in figure 5. 2, 3, and 4. Cross sections of the intestine containing semi-solid mass composed largely of bacterial cells of various sizes and shapes intermixed with food materials. 5. A more posterior section of the midintestine in which a mass of uniform rod-shope bacteria are found in a compact layer surrounding a homogeneous mass of food materials. 6. Immature eggs (approx. 25×). B. A more highly magnified view of the bacterial mass in the midintestine shown in A, 5, and in figure 8. (Approx. 400×.)

tinal tract. If the intestinal tract contained a large quantity of food material, the bacteria were usually numerous; if the intestines were virtually empty, the bacterial content was also low. In general, food materials and bacteria were more abundant in the females than in the males.

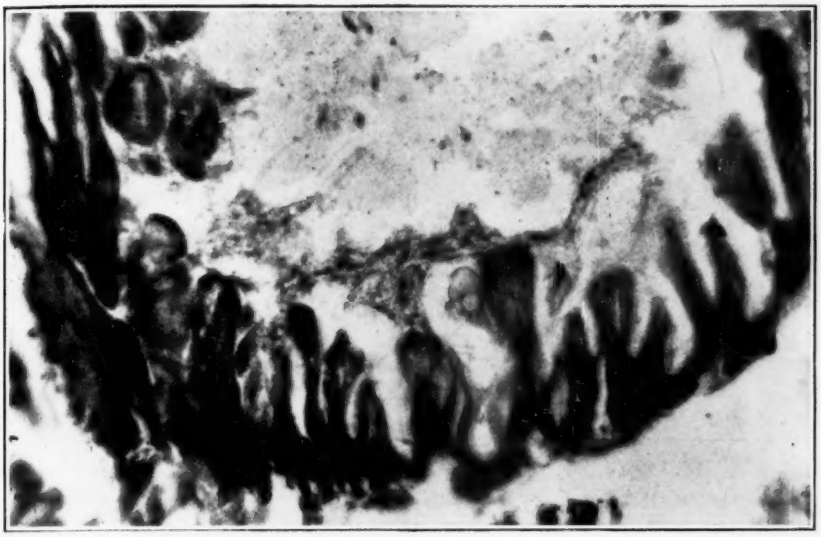

FIG. 8. Photomicrograph showing bacteria surrounding the homogeneous mass of food materials in the midintestine. A higher magnification of figure 7, A, 5 (approx. 200×).

The crop and the anterior portion of the midintestine usually contained a mixed culture of bacteria of all sizes and shapes mixed uniformly throughout the food material, presenting a picture very similar to that found in the intestines of the larva (Fig. 9). In many specimens there was some evidence of destruction and disappearance of the bacteria as they passed along the intestinal tract. The destruction, however, was incomplete and appeared to be somewhat selective in its action. By the time the food had reached the posterior portion of the midintestine all the bacteria had disappeared except a group of short rod-shape bacteria that had accumulated in a layer surrounding the homogeneous food material and lying between it and the walls of the intestine (Figs. 7, 8, 10, and 11). The walls of the midintestine are at this point lined with papillose epithelial cells. A careful examination under high magnification showed the bacteria in the space between the food contents of the intestine and a thin transparent peritrophic membrane. They were all short rods and were very uniform in size and shape. The regular arrangement of the cells in definite chains would appear to indicate that these bacteria were in a congenial

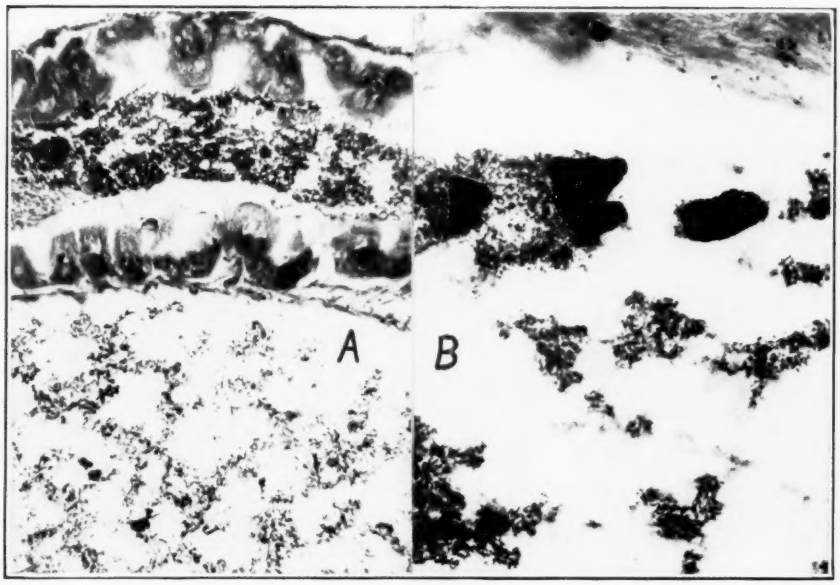

FIG. 9. Photomicrographs showing bacteria in crop and midintestine of the imago. A. Section showing bacteria in intestinal tract (at the top) and in the crop (at the bottom). (Approx. 100 ×.) B. Higher magnification of bacteria in the crop. (Approx. 350 ×.)

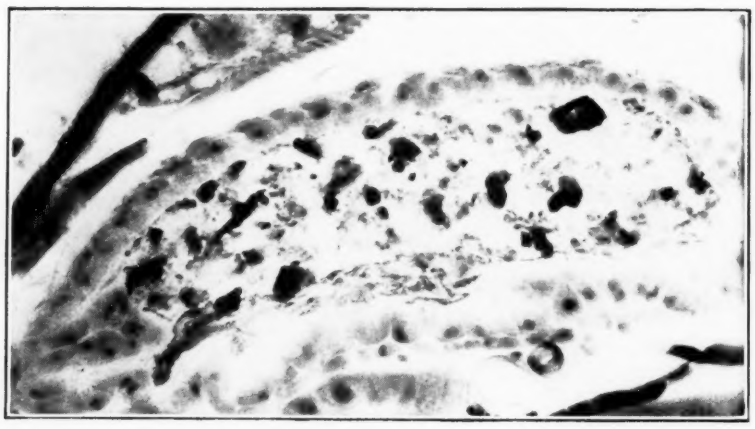

FIG. 10. Photomicrograph showing a section of the hind intestine with chains of rod-shape bacteria surrounding the granular residue of food materials. (Approx. 250 ×.)

FIG. 11. Photomicrograph showing a mass of short, rod-shape bacteria of uniform size between the granular residue of the food materials and the walls of the hind intestine. (Approx. 500×.)

environment and were actively growing and multiplying. From this point the food materials rapidly disappeared, but the short rod-shape bacteria could be traced throughout the length of the intestine to the anal opening (Figs. 12 and 13). These bacteria, as previously demonstrated culturally (6), pass out in the feces in a viable condition. In certain specimens very

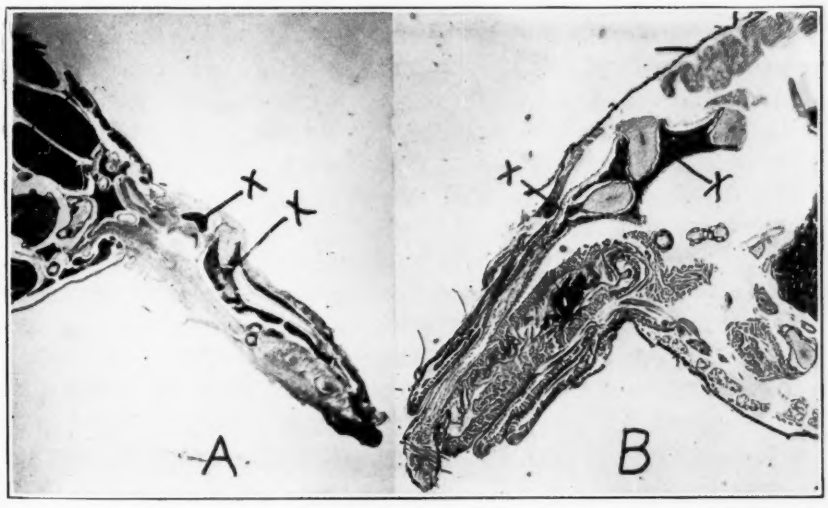

FIG. 12. Photomicrographs showing sections through the rectum and posterior end of the intestinal tract showing, at X, masses of apparently uninjured bacteria. A. Approx. 20×; B. Approx. 30×.

little food material was found in the intestines. In such specimens small numbers of the bacteria were nearly always found adhering in groups or chains to the peritrophic membrane (Fig. 14).

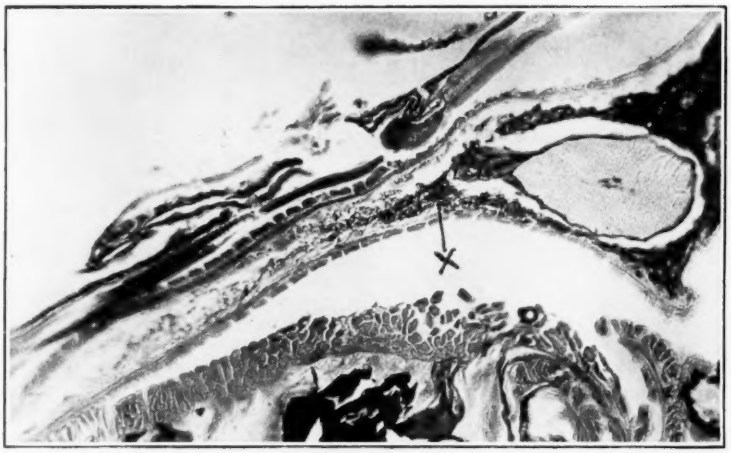

FIG. 13. Photomicrograph showing bacterial masses in the rectum and in passage leading to anal opening. The same section shown in figure 9, B. These bacteria are all short rods measuring about 0.5 microns x 1½ microns and are all apparently uninjured. They obviously are excreted in a viable condition. (Approx. 100×.)

It is of interest to compare the histological relationship between bacteria and the seed-corn maggot with that found in related insects by other workers. Petri (10) in 1910 published an account of his studies of the olive fly (*Dacus oleae*). He showed that a symbiotic relationship existed between this insect and several species of bacteria among which *Bacterium savastanoi* E. F. S. (the olive-knot pathogene) was almost universally present. He found the bacteria constantly localized in 4 spherical blind sacks arising from the forepart of the midintestine of the larva. They also were found at times in the lumen of the intestines and were frequently passed out with the excrement.

Petri showed that the bacteria survived during pupation and were present in the body of the imago upon emerging from the puparium. Shortly before pupation most of the bacteria were expelled by the larva but a few migrated to the forepart of the oesophagus. They were harbored here until a special sack-like outgrowth appeared above the pharynx, in which they developed in large numbers. After emergence of the imago the bacteria spread throughout the intestinal tract. In female flies they soon became located in a series of small glandular sacks near the anal opening. These glandular sacks were so situated that the eggs, on passing out of the ovipositor, became surface-contaminated with the bacteria. The

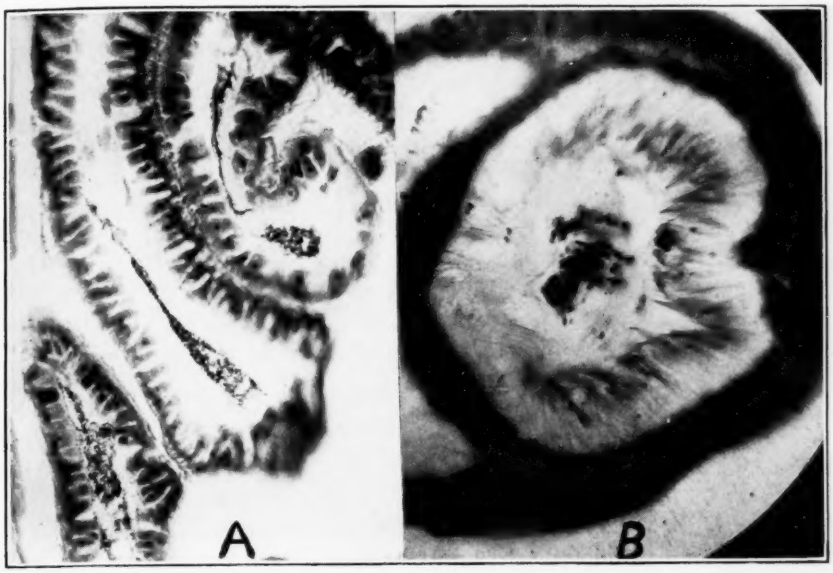

FIG. 14. Photomicrograph showing sections of mid- and hind-intestines containing very little food material but with masses of bacteria adhering to the peritrophic membrane. A. Midintestine. (Approx. 75 ×.) B. Hind-intestine near rectal valve. (Approx. 600 ×.)

bacteria multiplied rapidly in the gelatinous coating over the egg and migrated through the air canals around the micropyle, thus infecting the embryo. In this way the continued association of the bacteria and insect was insured.

Stammer (11) has recently confirmed the histological findings of Petri and has also investigated histologically numerous other representatives of the Trypetidae. He found symbiotic bacteria present in all of the representatives of this group that he studied. In some species the bacteria were harbored in special blind sacks off the intestines of the larvae, while in other species they occurred free in the intestinal tract. In some species special devices for insuring the contamination of the eggs were found in the adult female; in others no such structures were present. Where these structures occurred they were much simpler than those found in *Dacus oleae* consisting principally in the union of the anal tract and the vagina into one common opening.

In the imago of the seed-corn maggot no such structures were found, and, although the openings of the anus and vagina are very close together, no direct union was observed. Also, as pointed out above, the bacteria were not concentrated in special organs but were found throughout the intestinal

tract. The symbiotic relationship here, therefore, appears to be of a very simple type. This is probably what would be expected when the life habits of the insect are considered. Here the larvae spends its life in a mass of decaying plant tissue, surrounded by a constant supply of bacteria. The presence of such special organs for harboring the bacteria would appear superfluous. Likewise, the eggs being deposited in the soil, the larvae have abundant opportunity to become contaminated with the bacteria commonly associated with it. A special structure to insure contamination would appear of less significance in the seed-corn maggot than in those insects that deposit their eggs directly into plant tissues.

NUTRITIONAL STUDIES

Preliminary experiments reported in 1926 (6) showed that sterile larvae were unable to grow when placed on sterile nutrient agar or sterile potato plugs. If, however, bacteria were added to the medium, they would grow and pupate normally. Although these experiments demonstrated that the bacteria played an important rôle in the metabolism of the insect, no attempt was made to explain the exact relationship. Further studies have thrown some additional light on the problem. Since the publication of the paper mentioned above (6), Huff (4) has reported the results of his nutritional studies on the seed-corn maggot. He also found that sterile larvae would not grow to maturity on sterile beef-extract agar or potato plugs but that they would grow normally on contaminated agar or potato plugs.

Huff was unable to grow the larvae on bacteria-free filtrate from unheated potatoes or on sterile potato to which had been added a suspension of bacteria killed by heat. On the other hand, the larvae grew normally on potato plugs, beans, and peas that had been partially decomposed by bacteria and then sterilized by heat. He was able to grow the larvae to maturity on sterile germinating seedlings also. From these results he concluded that the bacteria, *per se*, are not essential to the development and pupation of the larvae but that, by their digestive action, they convert the plant tissue into available food for the larvae. The presence of available food in the sterile germinating seedlings is assumed to be due to the natural processes of digestion which occur when seeds germinate. Similar experiments have been made by the writer and the results obtained agree in all essential details with those of Huff and tend to bear out his general conclusions. One point of difference, however, should be mentioned. Huff states that the larvae grew rapidly and pupated in the normal length of time while feeding on sterile seedlings. In the writer's experiments, although larvae grew to maturity on sterile seedlings, they always grew more slowly than larvae on contaminated seedlings and a much lower percentage pupated normally.

DISCUSSION AND CONCLUSIONS

From the evidence presented here and in previous publications, it is possible to draw some general conclusions concerning the nature of the relationship between the seed-corn maggot, the blackleg disease of potatoes, and the associated bacteria. The evidence shows that the seed-corn maggot is a common pest of the potato plant. The adult female fly deposits its eggs on the seed piece or in the soil near the base of the plant. When the eggs hatch, the larvae attack the seed piece and at the same time inoculate it with a mixed culture of bacteria that cause a certain amount of decay. In some cases the bacteria may consist of only the saprophytic species that make up the normal soil flora and that find the potato tubers a favorable substrate for growth. These saprophytes are able to rot the tubers only when aided by the destructive action of the larvae. In other cases the blackleg pathogene may be included among the bacteria making up the inoculum. As pointed out previously by the writer (6), the blackleg pathogene can be introduced on the eggs of the insect or in the feces of the imago. Since it also has been demonstrated by the writer (7) and Patel (9) that the blackleg pathogene can survive in the soil, it is reasonable to assume that it can be inoculated into the potato seed piece by the larvae along with other bacteria from the soil. When the blackleg pathogene is introduced, other conditions being favorable, the plant will succumb to the disease.

As shown by the nutritional experiments of Huff (4) and the writer, the bacteria aid the larvae in their development by digesting the tissues of the potato, thus making them more available as food. In all probability the bacteria as such are not utilized as food by the larvae. This view is supported by the histological studies which show that the bacteria are not digested or destroyed but are passed through the intenstinal tract without any apparent injury.

When the larvae pupate some of the bacteria among which they are feeding apparently are retained within the puparial case and are to be found in and on the body of the imago as it emerges. This has been demonstrated repeatedly by cultural methods, although the histological details have not been satisfactorily determined. If the larva has fed in a potato plant affected with blackleg, it is only reasonable to expect the blackleg pathogene to be present in the puparium and in the imago. This has been shown true by numerous cultural and inoculation experiments (6). It has been demonstrated also that the adult flies feed upon liquids containing bacteria and that bacteria, including the blackleg pathogene, are frequently found in the intestinal tract and can be passed out in a viable condition in the feces.

For practical considerations it should be recognized that the dissemination of the pathogene is incidental from the standpoint of the insect.[4] Although some kinds of bacteria are always associated with the insect in various stages of development, the presence of the blackleg pathogene is not essential. However, if the insect has fed on tissues affected with the disease, the pathogene is likely to be present. It should not be assumed that blackleg will necessarily develop in every plant attacked by the insect. If the pathogne is not introduced with the eggs and if the larvae do not introduce it from the soil there is no reason to expect the disease to develop. When the pathogene is present, on the other hand, the larvae serve as effiicient agents of inoculation and aid infection and development of the disease.

When these facts are considered, it is obvious that the origin of flies which deposit their eggs upon a potato plant may determine the extent to which they disseminate the pathogene. Flies arising from larvae that have developed in blackleg plants are more likely to have the blackleg pathogene in their system than those from larvae developed in other decaying organic matter. Numerous isolations from flies from various sources have tended to verify this assumption. There is also some evidence to show that this insect is a more frequent carrier of the disease in regions where potatoes are intensively grown.

SUMMARY

1. A comparative study has been made of the internal bacterial flora of the seed-corn maggot, the principal bacteria associated with the blackleg disease of potatoes, and certain soil-inhabiting bacteria. Several species occurring in each of these groups were found identical. The blackleg pathogene was obtained from each of these three groups, although certain soil saprophytes, including *Pseudomonas fluorescense* (Flügge) Migula and *Ps. non-liquefaciens* Eisenberg, appeared to predominate.

2. It is concluded that the kinds of bacteria associated with the insect are determined largely by the nature of the material on which it feeds. Flies that have developed in potato plants affected with blackleg would be more likely the carriers of the pathogene than those developing in organic matter destroyed by the common soil saprophytes.

3. Histological studies of the relationship between the seed-corn maggot and bacteria are reported. These studies indicate that bacteria of many species pass uninjured through the intestinal tract of the larvae. The

[4] In an abstract of this paper (Phytopath. 20: 127, 1930) the insect was termed an "occasional" carrier. "Incidental" is preferable, inasmuch as occasional often conveys the idea of infrequency, while incidental does not involve the question of frequency. The dissemination of the blackleg pathogene by the seed-corn maggot is always incidental, although it occurs frequently.

larvae apparently do not feed upon the bacteria as such. Efforts to follow the course of the bacteria in the puparium during metamorphosis were unsuccessful. In the imago, also, bacteria were found to pass through the intenstinal tract without injury. Here it appeared that certain species of bacteria were destroyed and digested by the insect, while certain short rod-shape species were uninjured and apparently grew and multiplied in the posterior portion of the tract.

4. In neither larva nor imago were the bacteria found to be harbored in special organs but were constantly present in the lumen of the intestines. In the larva bacteria were present in the proventriculus or enlarged portion of the midintestine surrounding the oesophageal valve. In the imago the bacteria were present in the crop and intestinal tract. In the crop and a portion of the midintestine the bacteria were scattered throughout the food material, but in the more posterior portions of the intestinal tract they were accumulated in a layer between the food mass and the peritrophic membrane. Their arrangement in definite chains indicated that growth and multiplication had occurred in this location.

5. No special devices for insuring contamination of the eggs were observed. The histological relationship between bacteria and this insect are considered to be of a simpler type than most of the Trypetidae studied and described by Stammer (11).

6. Nutritional studies with the larva of the seed-corn maggot indicate that bacteria aid its development by transforming the plant tissues into a form more readily assimilated. The ability of the larva to grow normally on seed partly decayed by bacteria and then sterilized by heat, as well as on sterile germinating bean seed, indicates that the bacteria are not utilized as food by the insect but that they digest the plant tissues and make them available as food for the larva.

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THE LIFE HISTORY OF SCLEROTINIA SCLEROTIORUM WITH REFERENCE TO THE GREEN ROT OF APRICOTS

RALPH E. SMITH

One of the most characteristic fungus diseases of central California is the so-called green rot of apricots. It is characteristic in its sporadic occurrence, breaking out locally in virulent epiphytotic form and causing almost complete crop losses in certain seasons, then disappearing entirely for a period of several years. The first recorded instance of this kind was in 1910 when the apricot crop in the district about the southern end of San Francisco Bay was greatly reduced by this disease. In 1916 and 1928 further epiphytotics occurred. Since then no outbreak of green rot has taken place in that district. Since 1910 there has been a considerable planting of apricots in the San Joaquin Valley, mainly in Stanislaus and Merced counties, of the late, slow-blooming Tilton variety. In 1928 and several preceding years losses from green rot were severe in this district.

FIG. 1. Green rot of apricot. Infection of young fruit through dried calyx by *Sclerotinia sclerotiorum*.

The disease consists in a complete or partial destruction of the young fruit, commencing while it is still in the "jacket" stage (enclosed in the

dry calyx (Fig. 1). It is entirely distinct from the blossom-blight form of "brown rot" which is caused by *Sclerotinia cinerea* (Bon.) Schr. in that it attacks the young fruit through the dead calyces rather than the living petals of the blossom (Fig. 2). The tree is not ordinarily attacked by this disease in any part except the blossom and the young fruit. In 1928 almonds were quite generally attacked in the same manner as the apricot.

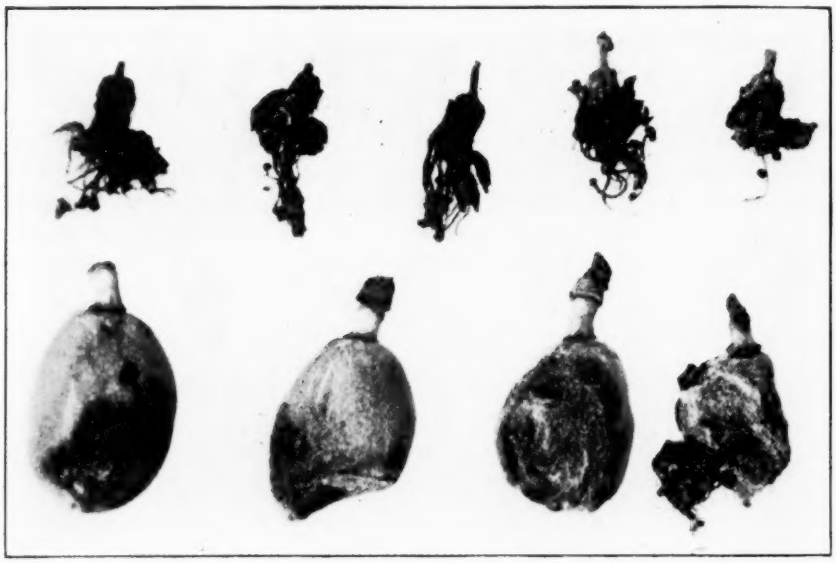

FIG. 2. Young apricots (below) attacked by *Sclerotinia sclerotiorum* through dead calyx, contrasted with blossom-blight effect of *S. cinerea* (above).

The idea is generally accepted that the cause of this disease is the common cottony mold fungus, *Sclerotinia sclerotiorum* (Lib.) Mass. A fungus of this type may be regularly and easily cultured from affected fruit. The typical mycelium and sclerotia develop profusely from such material either in a moist chamber (Fig. 3, A) or, in wet weather, on moist soil in the orchard. The fungus readily attacks the young fruit in the typical manner when inoculations are made either with mycelium or ascospores. Typical apothecia develop from the sclerotia (Fig. 3, B). This has been observed in the soil under apricot trees on sclerotia kept in moist sand and even in flask cultures (Fig. 3, C).

Sclerotia have never been observed upon the tree; in fact, the fungus makes almost no visible development at the points of attack. The young fruits are killed and drop to the ground or may even resist the attack sufficiently to develop to maturity marked with a scar or lesion, as in figure 2.

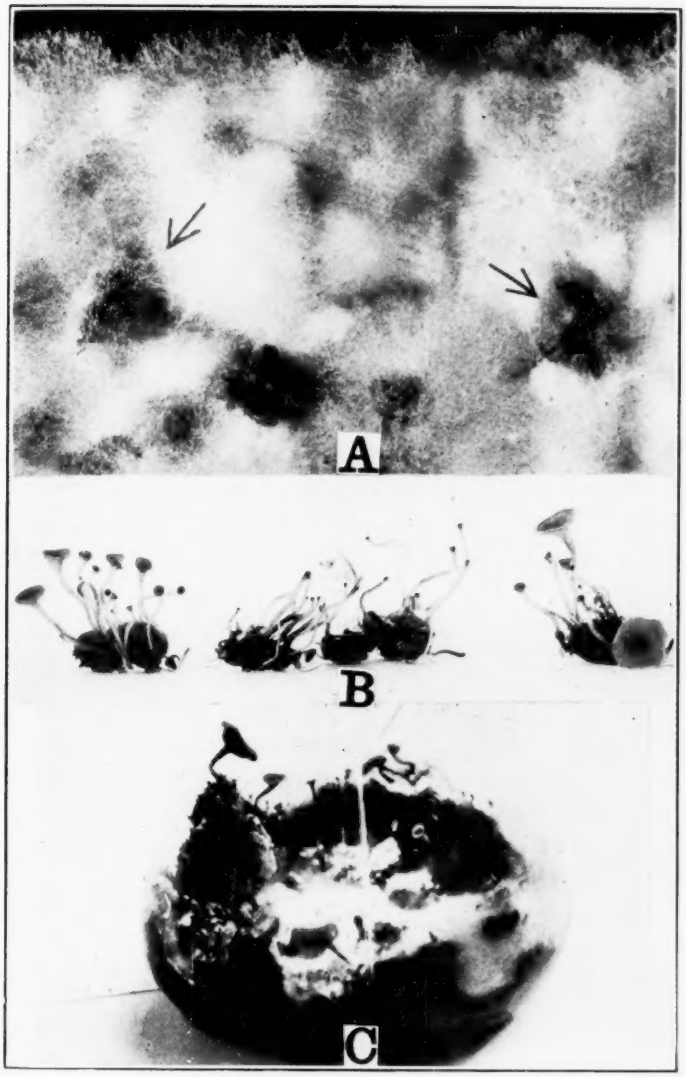

FIG. 3. A. Mycelium of *Sclerotinia sclerotiorum* from young apricots and blossoms after 2 days in moist chamber. Conidiophores of *Botrytis cinerea* also present at arrows. B. Sclerotia and apothecia of *S. sclerotiorum* from soil in apricot orchard. 1916. C. *S. sclerotiorum* producing apothecia from sclerotia in flask culture. 1916.

Any further development of the fungus and, particularly, so far as known, the formation of any spore stage take place only in the soil. The apricot and more rarely the almond (Fig. 4) are the only known hosts of this type of disease. Although, as shown in table 1, spores of the fungus are abundant on flowers of the cherry, prune, and peach, and many annual plants, no natural infection of these hosts in the manner of aerial attacks of the blossoms has ever been observed.

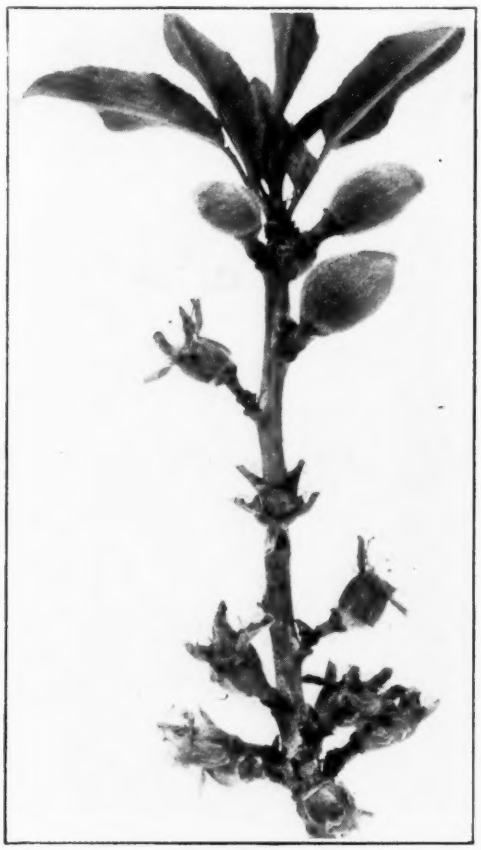

FIG. 4. Young fruits of almond in jacket, when infection with *Sclerotinia sclerotiorum* takes place.

Nixon and Curry (2) state that they produced typical green rot of apricots by inoculation with spores of *Botrytis cinerea* obtained by culturing the diseased fruit and think that the disease may be caused by various fungi, including Botrytis and Sclerotinia. Smith and Smith (5, p. 1097) expressed the same idea, stating that green rot is "caused by vari-

ous fungi of which perhaps a species of Sclerotinia, apparently *S. libertiana,* is most common, causing a decay of the young fruit on the tree. When such fruit is picked and placed in a moist chamber it develops an abundant cottony mold in which black sclerotia soon form. . . . *Botrytis vulgaris* is also common in this trouble."

C. O. Smith (3) describes a twig and branch infection of lemon trees caused by *Sclerotinia libertiana* in which "the twigs may become infected through the blossoms or through some injury. The disease is often worse following a cool winter when the tissue is weakened or injured by frost." A similar infection is quite common on frost-injured fig trees in the interior valleys of California. Botrytis also is abundant on such branches and it too is mentioned by Smith as being associated with the lemon disease.

These diseases of the apricot, almond, lemon, and fig are exceptional among the troubles attributed to *Sclerotinia sclerotiorum* in that they attack the aerial parts of the host in a manner which precludes soil-borne

FIG. 5. Apothecia of *Sclerotinia sclerotiorum* in soil beneath vetch cover crop in lemon orchard (after C. O. Smith).

infection by direct mycelial contact. Most diseases caused by this fungu are of the nature of stem or fleshy-part rots originating in the latter manner. In the apricot and almond infection occurs uniformly all over the tree up to a height of many feet above ground. Since in these hosts the fungu attacks only the blossoms and young fruit and has no conceivable method of holding over on the tree in mycelial form, it is the common supposition that infection must originate from spores and that the inception of the disease is due to ascospores originating in apothecia from sclerotia in the soil beneath the tree. Such apothecia, together with the mycelium and sclerotia of the fungus, can actually be found at times in apricot and lemon orchards, particularly where there is a heavy growth of cover crop, weeds, or other vegetation (Fig. 5). In line with this idea advice has commonly been given to apricot growers that orchards be plowed or cultivated before the end of the blooming period in order to break up the surface soil, disturb the growth of the fungus, and prevent the formation of apothecia.

EXPERIMENTAL WORK

As a basis for the successful control of the disease on apricots it seemed desirable to determine the time when the fungus first actually reaches the affected parts. Growers report the complete failure of spraying with any material or at any time to control this disease. The efforts heretofore made consist mainly in the usual spraying for brown rot (Bordeaux mixture in the early blooming stage) together with later applications at the end of the bloom or in the jacket stage (dead calyx still attached to the young fruit). The latter seems the most logical time, the infection of the young fruit very evidently takes place through the dead calyces; it has frequently been demonstrated that the removal of these dried jackets by shaking or hand picking completely prevents infection.

Method.—To determine the time when the fungus first reaches the tree the following method was adopted: blossoms, young fruit, foliage, or other parts to be tested, were gathered at intervals throughout the supposed infection period. Each sample was placed in a separate paper bag, taken at once to the laboratory, and put into a moist chamber on wet filter-paper. The material was left until fungus colonies developed and when any which looked like *Sclerotinia sclerotiorum* appeared transfers were made to culture tubes for positive identification.

The work was started in March, 1929, in the large peach and apricot orchard belonging to the California Packing Corporation, near Merced. The previous year (1928) the Tilton apricots in this orchard had been an almost total loss from green rot.

The first material was collected on March 14, 1929. Up to this time the winter, in general, had been cold and dry and the ground in this orchard

had been very dry until rain fell on March 5 and 12. The soil was covered with an abundant growth of bur clover. The Tilton apricots were just past full bloom. In the majority of the blossoms the very young fruits were enclosed in the calyces with some petals still attached. The trees had been sprayed with Bordeaux mixture about March 2. A careful search revealed no trace of sclerotia or apothecia or mycelium on the soil or vegetation beneath the trees. Comparatively cold, dry weather prevailed during the spring with much frost damage to young fruit.

Table 1 shows that *Sclerotinia sclerotiorum* developed abundantly in moist chamber on apricot and almond material from Merced and Stanislaus counties and on the cover crop from beneath the trees. After observing this the search was widened to include other localities in central California and a variety of host material. Flowers and leaves of various fruit trees and native plants from many different places were collected and placed in moist chambers with the result that *S. sclerotiorum* appeared as one of the commonest fungi occurring upon them. In no case was any development of the fungus visible when the material was gathered and it seemed certain that the organism must have been present in spore form. The original growth was obtained in almost every case only on petals, probably because they formed the most favorable growth medium. Botrytis, apparently of more than one species, was abundant on much of the material but was readily separated from *S. sclerotiorum* in isolation cultures. Figure 3, A, shows typical mycelial growth of *S. sclerotiorum* in the moist chamber coming from young apricot fruit and blossoms with 2 colonies of Botrytis

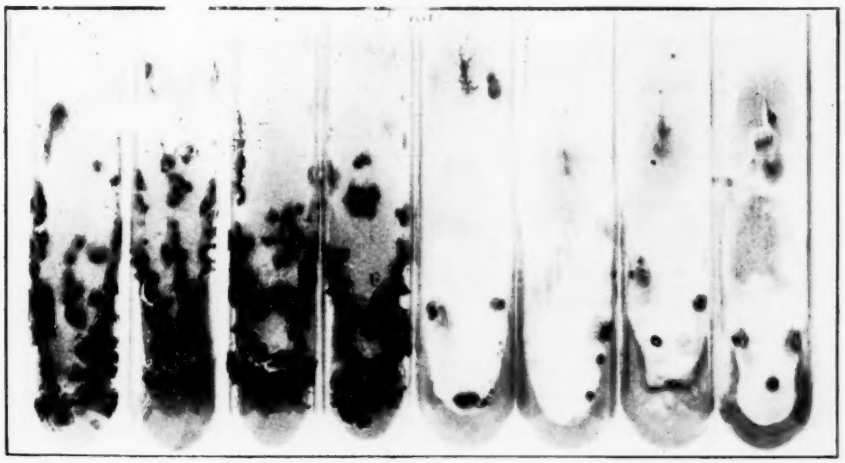

FIG. 6. Cultures of Botrytis (left) and *Sclerotinia sclerotiorum* (right) from young apricots with green rot.

TABLE 1.—*Results of moist chamber tests of apricot and other material for Sclerotinia sclerotiorum*

Lab. no.	Date collected	Locality	Host	Part	Results
50	3/14/29	Merced	Apricot	Flowers	3 isolations Botrytis, 1 *S. sclerotiorum*
51	"	"	Soil and dead leaves from apricot orchard		2 isolations *S. sclerotiorum*, 2 Botrytis
52	"	"	Soil and dead leaves from apricot orchard		No *S. sclerotiorum*
53	"	"	Bur clover[a] in apricot orchard	Green leaves	Botrytis and *S. sclerotiorum* in separate colonies
54	3/15/29	Oakdale	Almond	Flowers	No *S. sclerotiorum*, Botrytis abundant
55	"	"	Soil and dead leaves from apricot orchard		No *S. sclerotiorum*
61	3/18/29	Modesto	Apricot	Flowers	*S. sclerotiorum* abundant
62	3/19/29	Oakdale	Almond	"	No *S. sclerotiorum*, some Monilia
63	3/21/29	Merced	Green undergrowth and dead leaves from apricot orchard		Colonies of Botrytis and *S. sclerotiorum*
64	"	"	Apricot	Flowers	*S. sclerotiorum*
65	"	"	Almond	"	Botrytis and *S. sclerotiorum*
66	3/27/29	Berkeley	Apricot	Young fruits with jackets	Abundant and almost pure growth of Botrytis, no *S. sclerotiorum*
67	"	Yountville	French prune	Flowers	Botrytis and *S. sclerotiorum* abundant
68	"	"	Pear	"	*S. sclerotiorum* doubtful, much Rhizopus
69	"	Napa	Green chickweed[b] from roadside		*S. sclerotiorum*
70A	"	"	Cherry	Flowers	" "
70B	"	"	Pear	"	No *S. sclerotiorum*, much Rhizopus
70C	"	"	Rape	"	*S. sclerotiorum*
75	3/29/29	Merced	Peach	"	" "
76	"	"	Apricot	Young fruits with jackets	" "

[a] *Medicago hispida* Gaertn.
[b] *Stellaria media* (L.) Cyr.

TABLE 1.—*Continued*

Lab. no.	Date collected	Locality	Host	Part	Results
78	3/29/29	Merced	Flowering peach	Flowers	*S. sclerotiorum* very abundant
79	"	"	Peach	"	*S. sclerotiorum*
80	"	"	"	"	Botrytis
81A	"	"	Plum	"	*S. sclerotiorum*
81B	"	"	Apricot	Young fruits with jackets	" "
85	4/3/29	Modesto	"	Young fruits with jackets	No *S. sclerotiorum*
86	"	"	"	" " " " [d]	No *S. sclerotiorum*
87	"	"	"	" " " " [e]	Vigorous growth of *S. sclerotiorum*
88	"	"	Almond	" " " "	Much *S. sclerotiorum*
89	"	Oakdale	"	" " " "	Some Monilia, no *S. sclerotiorum*
90	"	"	"	" " " "	Some *S. sclerotiorum*
94	4/5/29	Berkeley	Crab apple	Flowers	No *S. sclerotiorum*, some Botrytis
98A	4/6/29	"	Cherry	"	" " " " "
98B	"	"	Plum	"	" " " " "
100	4/8/29	"	Barberry	"	" " " " "
101	4/10/29	Sebastopol	Apple and pear	"	No *S. sclerotiorum*, much Rhizopus
102	"	Petaluma	Annual wild flowers		*S. sclerotiorum* very abundant
106A	4/11/29	Merced	Apricot	Young fruits without jackets	*S. sclerotiorum*
106B	"	"	"	Jackets from last	No *S. sclerotiorum*, Botrytis abundant
108	"	"	Green bur clover from apricot orchard		*S. sclerotiorum*
109	"	Morganhill	French prune	Flowers	" "
110	"	Merced	Peach	Young fruit	No *S. sclerotiorum*
115	4/18/29	Brentwood	Apricot	Young fruit killed by frost	*S. sclerotiorum*
116	4/19/29	Berkeley	"	Young fruit	No *S. sclerotiorum*, much Botrytis
117	4/20/29	"	Cherry	Young fruit in jackets	No *S. sclerotiorum*
130	5/18/29	Merced	Apricot	Young fruit and leaves	" " "
131	"	"	"	Young fruit from ground	" " "

[c] Sprayed with Bordeaux mixture and oil in jacket stage.

[d] Sprayed with lime sulphur in jacket stage.

[e] No spraying.

TABLE 1—*Continued*

Lab. no.	Date collected	Locality	Host	Part	Results
132	5/18/29	Merced	Green bur clover from apricot orchard		*S. sclerotiorum* abundant
133	"	"	Dead and moldy bur clover from apricot orchard		No *S. sclerotiorum*
134	"	Delhi	Wild primrose[f]	Flowers	" " "
135	"	"	Wild lupine[g]	"	" " "
136	5/24/29	Rocklin	California poppy[h]	"	" " "
141	6/19/29	Sebastopol	Native wild plants	"	" " "
142	"	Napa (same as 69)	California poppy	"	" " "
144	6/24/29	Modesto	Peach	Fruit deformed by frost	No *S. sclerotiorum*, white mycelium of Fusarium
165A	2/12/30	Merced	Apricot	Twigs with unopened buds	No *S. sclerotiorum*
165B	"	"	Green grass from apricot orchard		" " "
165C	"	"	Dead leaves and grass from apricot orchard		" " "
165D	"	"	Almond	Flowers and buds	" " "
166A	2/22/30	Atascadero	"	Flowers	" " "
166B	"	Morganhill	"	"	Much *S. sclerotiorum*
166C	"	"	Weeds in prune orchard		No *S. sclerotiorum*
167A	2/28/30	Turlock	Apricot	Flowers	*S. sclerotiorum*
167B	"	Byron	Yellow flowered annual composite	Whole plant with flowers	No *S. sclerotiorum*
167C	"	Merced	Apricot	Flowers	*S. sclerotiorum*
167D	"	"	"	"	" "
167E	"	"	"	"	" "
167F	"	"	"	"	" "

[f] Oenothera.

[g] Lupinus.

[h] *Eschscholtzia californica* Cham.

TABLE 1—*Continued*

Lab. no.	Date collected	Locality	Host	Part	Results
167G	2/28/30	Escalon	Apricot	Flowers	*S. sclerotiorum*
167H	"	Oakdale	Almond	"	" "
168A	3/7/30	Morganhill	Melilotus	"	" "
168B	"	"	Wild radish[i]	"	" "
168C	"	"	Almond	"	" "
168D	"	"	Mustard[j]	"	" "
169A	3/22/30	Collegeville	"	"	" "
169B	"	Turlock	California poppy	"	" "
169C	"	"	Apricot	"	*S. sclerotiorum* abundant
169D	"	Atwater	Peach	"[k]	" " "
170A	4/1/30	Napa	California poppy	Flowers	" " "
170B	"	"	Prune	"	" " "
171A	4/4/30	Merced	Apricot	Dried jackets	*S. sclerotiorum* abundant on every calyx
171B	"	Escalon	"	Leaves	No *S. sclerotiorum*
172	4/14/30	Berkeley	Cherry	Calyces	No *S. sclerotiorum*, much Botrytis
173	4/29/30	Merced	Mustard	Flowers	No *S. sclerotiorum*

[i] *Raphanus sativus* L.

[j] *Brassica campestris* L.

[k] Sprayed with Bordeaux in bud.

beginning to appear. This photograph was taken 48 hours after the material was put into the moist chamber.

Typical cultures of the 2 fungi Botrytis and *Sclerotinia sclerotiorum* are shown in figure 6. Botrytis is grayish brown with typical conidia production (varying in abundance) and thin membranous sclerotia inseparably attached to the medium. Sclerotinia shows pure white mycelium, total absence of conidia, and round meaty sclerotia separating cleanly from the substratum. *S. sclerotiorum* was never obtained on material from Berkeley (Nos. 66, 94, 98A, 98B, 100, 172). The number and kind of samples tested appeared sufficient to give some significance to this result. Flowers of pear and apple (Nos. 68, 70B, 101) never yielded *S. sclerotiorum* but always a great abundance of Rhizopus. It does not appear possible, however, that any host specificity as to the occurrence of the fungus could have existed in light of its universal distribution on other material in the same vicinity. It is more probable that certain materials were more favorable than others to the growth of the fungus, even though the spores were present on all. Mustard blossoms and flowers of the California poppy (Eschscholzia) seemed to furnish a particularly favorable medium since such material from different localities usually developed an abundant growth of *S. sclerotiorum*. The various specimens of these plants were gathered in all sorts of places, orchards, alfalfa fields, and bare land, and along roadsides.

The fungus was not obtained from fruit trees after the latter part of April, when it began to become less abundant, but was recovered from green bur clover on May 18. This as usual was on sound, green leaves which showed no visible sign of infection before being placed in the moist chamber.

No rain fell during May but there was considerable precipitation during the first half of June. The last test was on June 24. Not a single case of green rot or any visible natural development of *Sclerotina sclerotiorum* was observed in 1929.

In 1930 the work was reopened on February 12. Almonds were in bloom, but on the Tilton apricots in the Merced orchard the flowers had not yet opened. No cover crop had been planted in this orchard and the ground had recently been cultivated so that it was completely bare of vegetation, except a little grass and dead leaves around the base of each tree. No rain had fallen for two weeks and the soil was quite dry. No sign of the mycelium, sclerotia, or apothecia of *Sclerotinia sclerotiorum* could be found beneath the trees and the conditions were entirely unfavorable for any such development. The first sample taken for culturing consisted only of twigs and buds, no blossoms having opened. It could hardly be expected that the fungus would develop upon such material even if spores were

present. The almond blossoms, however, gathered on the same day in the same locality, constituted a very favorable medium, but these did not develop the typical organism. Almond blossoms gathered at Atascadero (San Luis Obispo County) on February 22 did not develop the fungus, but similar material collected the same day at Morganhill (Santa Clara County) gave a very abundant growth. This came from an orchard with perfectly bare, dry soil and with conditions in every way most unfavorable to the growth of *S. sclerotiorum* in the ground under the trees. Commencing February 28 and continuing until April 4, a series of 18 samples representing a wide variety of material growing under various conditions in Merced, Stanislaus, San Joaquin, Contra Costa, Napa, and Santa Clara counties did not fail to develop an abundance of the fungus in any instance. In the case of sample No. 168A (from a heavy growth of Melilotus under prune trees at Morganhill) *S. sclerotiorum* was growing quite abundantly in places on the dense mass of vegetation in the orchard. This was the only case during this entire investigation where visible growth of the fungus was found under natural conditions. Even here, however, perfectly sound, individual, separate leaves taken at a height of 2 or 3 feet from the ground developed *S. sclerotiorum* almost as quickly and abundantly as did foliage from the thick mat of growth near the soil where the fungus growth was visible in the orchard. This indicated the presence of spores distributed abundantly all over the vegetation as well as the contact mycelium near the ground. No apothecia were found in this orchard.

No case of green rot was found during 1929 and 1930. No apothecia were found in the soil, and, with the exception just noted (No. 168A), no natural infection with *Sclerotinia sclerotiorum* on any host was observed.

Note: In 1931 moist-chamber testing of blossoms was commenced on January 20 and continued to March 10. It was a season of humid, mild weather and the fungus *S. sclerotiorum* was found to be universally present in extreme abundance on all sorts of vegetation in central California from Napa to Merced County. Flowers of fruit trees and annual plants were apparently covered with spores and the fungus developed profusely in almost pure culture whenever such material was put into moist chambers. No such development occurred, however, on the flowers in nature and the presence of the fungus would never have been suspected without the moist-chamber test.

During the current season apothecia of *S. sclerotiorum* were found in abundance, developing from old sclerotia in the soil among thick vegetation, particularly in fields of yellow mustard, *Brassica campestris* L. This is one of the commonest winter-blooming plants in central California and covers

thousands of acres of fields, vineyards, and orchards with solid sheets of yellow. From this season's experience it would appear that such fields of this or other favorable vegetation form the breeding ground of the fungus. Mycelium and sclerotia develop each year during the latter part of the winter on the plant remains in the soil. The sclerotia remain over summer and start to develop apothecia with the next winter's rains, thus producing successive crops of ascospores which fill the air of the whole vicinity. Whether the vegetation upon which the fungus develops and upon which the production of apothecia and ascospores depends is directly beneath the trees or at some distance is of little consequence.

DISCUSSION

The foregoing results indicate that:

1. In the green rot of the apricot and almond, infection with *Sclerotinia sclerotiorum* must take place by means of spores.

2. The production of such spores has no relation to the soil directly beneath the trees, the presence or absence of a cover crop, or similar conditions.

3. The fungus has no specificity for or limited occurrence on the apricot, almond, or other hosts on which infection may occur, but in the early part of 1929 and 1930 was present in great abundance on all sorts of vegetation.

4. The abundant presence of spores of *S. sclerotiorum* on vegetation has no correlation with the development of the fungus on vegetation or soil directly beneath.

5. Spore production and distribution must commence as early as February, previous to the time when growth of the fungus on soil and surface vegetation occurs. Spores must continue to be produced and distributed throughout the spring, since much of the material tested in the latter part of the season had not yet developed in the earlier part.

6. The distribution of the fungus is not effected by ascospores produced in easily found apothecia in the soil in close proximity to the final resting place of the spores.

The whole situation indicates the occurrence of some spore form of *Sclerotinia sclerotiorum* which is very abundantly produced and generally distributed throughout the spring season, regardless of whether conditions are favorable for the germination of spores and the development of a disease like green rot. In two seasons, at least (1929 and 1930), when no green rot appeared and almost no development of *S. sclerotiorum* on annual vegetation was observed (sample No. 168A being the only exception), the fungus was universally present when given an opportunity to develop in a moist chamber.

Relation to Botrytis: The fact that Botrytis is so commonly associated with *Sclerotinia sclerotiorum* in almost all cases of its occurrence and has even in some cases (Nixon and Curry (2), Smith and Smith (5)) been thought to be the cause of green rot raises the old question of the possibility of this being a conidial stage of *S. sclerotiorum*. If this were true the whole question under discussion would be solved. Gäumann (1, p. 325) says: "The economically important representatives of the subgenus *Eusclerotinia*, as *S. Fuckeliana*, *S. Libertiana*, *S. Trifoliorum* and the various Sclerotinias on monocotyledonous bulbs, in their choice of hosts are much less specialized than those of the subgenus *Stromatinia* . . . . During the summer they kill the infected organs, developing in them the sclerotia in which they over-winter. Their conidia arise singly on characteristically formed conidiopores and hence, in contrast to those of *Stromatinia*, are placed in *Botrytis* rather than in *Monilia*. As the cultural determination of the perfect form is rarely possible, one generally places them, especially those of the *S. Fuckeliana* and *S. Libertiana* groups, in the collective species *Botrytis cinerea*." In the same connection these authors reproduce an illustration of Botrytis by the writer with the legend "*Sclerotinia sclerotiorum*. Conidial stage *Botrytis cinerea* (after R. E. Smith, 1900)." Inasmuch as this drawing was published in an article attempting to prove that Botrytis is *not* the conidial stage of *S. Libertiana* (*sclerotiorum*), the inference is a somewhat ambiguous one.

In the investigation here reported no difficulty was ever experienced in definitely separating Botrytis and *Sclerotinia sclerotiorum* in culture, as stated on page 413 and illustrated in figure 6, even though their simultaneous occurrence here was typically common. The writer has, furthermore, been unable to find any literature or experimental evidence to substantiate the statement of Gäumann or to disprove the writer's own conclusion, published in 1900, that "*Sclerotinia libertiana* and *Botrytis cinerea* have no connection whatsoever with each other, and that the former species has no conidial stage of this type." There appear, therefore, to be no grounds for accepting the attractive hypothesis that the distribution of *S. sclerotiorum* takes place by means of a conidial stage of the Botrytis type.

Microconidia (spermatia, pycnospores): There is occasionally found, on the mycelium or sclerotia of *S. sclerotiorum*, an abundant production of minute, acrogenous microconidia. These at times have been seen to germinate and make a feeble growth. That they have any important function, however, in the reproduction of the fungus is extremely doubtful.

Ascospores: The usual conception of the life history of *S. sclerotiorum* is that already indicated, that the sclerotia, formed one year, lie over winter and reproduce the fungus the following year by the development of

ascospores or mycelium. In the case of the lemon the matter is well expressed by C. O. Smith (3, p. 245) as follows: "The vegetative stage is the white, cotton-like growth. . . . This growth has no spores, but can spread rapidly and has the power to infect . . . by . . . contact. . . . In this white growth, numerous black bodies known as sclerotia are developed. . . . After these bodies have gone through a period of rest and the favorable rainy, cool weather of the autumn and winter has come, they revive and become active. The sclerotia may then send out a fresh growth of mycelium, the vegetative stage, which could easily infect dead organic matter or the weaker plant growth of weeds or cover crops. Here after a time new sclerotia are again formed. From the sclerotia in the soil the spore stage (ascospore) is formed under the cover crops in the lemon orchards. . . . Some of these spores are carried by the wind to the fruit and twigs . . . where under favorable conditions they germinate and produce the vegetative stage. The apothecial stage in California begins to appear about the first of October and continues to be formed during the winter and spring. After the cover crops of the orchards are plowed under and cultivation begins, few apothecia (spore stage) are thought to be produced, for this stage the fungus requires moist, shaded conditions, such as are best found under cover crops during the rainy season."

On the basis of present knowledge the dissemination of wind-borne ascospores from sclerotia in the soil furnishes the only explanation of the distribution of this fungus on the aerial parts of plants. To account for the origin of such ascospores, in such abundance and general distribution as have been shown to be the case, it seems necessary to seek some more obscure and distant source than the soil beneath the trees or plants.

SUMMARY

The "green rot" disease of apricots and almonds, an infection of young fruits through adherent, dried calyces, occurs in a very sporadic manner in central California.

The usual cause of the disease is the fungus *Sclerotinia sclerotiorum*. *Botrytis cinerea* Pers. is commonly associated with affected material and, in some instances, has been considered to be the cause of the disease.

The common supposition has been that infection is caused by wind-borne ascospores originating in the soil beneath the trees from sclerotia produced by the fungus growing on cover crop or other surface plants.

Moist-chamber tests of blossoms of apricot and other fruit trees, as well as those of many annual plants, from many places in central California, showed that from about February 15 to June 15 this fungus was universally present on vegetation, presumably in some spore form. This was true

during two seasons (1929 and 1930) when no green rot appeared, when no development of *Sclerotinia sclerotiorum* (save in one minor instance) on surface-growing plants was observed, and in situations where vegetative growth of this fungus was extremely unlikely.

No evidence was found to suggest that *S. sclerotiorum* possesses a conidial stage of Botrytis or any other type.

It would appear that the aerial distribution of this fungus is much more general than has hitherto been suspected.

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PLANTS AFFECTED BY FIRE BLIGHT[1]

H. E. Thomas and H. E. Thomas

The plants known to be affected by fire blight, caused by *Bacillus amylovorus* (Burr.) Trev., already comprise an imposing group and have been reviewed by Snow (7), Rosen and Groves (6), and Pierstorff (4). At first glance it might appear that the list for North America is fairly complete. The cultivated plants of California, however, include a number of rosaceous trees and shrubs, some of them recently introduced, which do not seem to have been studied in relation to their susceptibility to fire blight.

It has been observed by various workers in California during the past several years that species of Cotoneaster and Pyracantha are affected by a disease assumed to be fire blight, although the writers have found no published record of experimental proof for any species of these genera save the early report by Arthur (2) from New York for *P. coccinea*.[2,3]

The work reported in this paper was initiated primarily to study the susceptibility of species of Pyracantha and Cotoneaster and was later expanded to include a number of other readily available plants. A few previously tested plants were included and certain others for which the evidence seemed inadequate. A number of inoculations were made on potted plants in the greenhouse at Berkeley, California, during the winter and spring of 1929–30. The remainder were field-inoculation tests at the University of California Deciduous Fruit Station, San José.

Of the plants used in the field, the species of Cotoneaster, Heteromeles, Kerria, Photinia, Pyracantha, Raphiolepis, and Spiraea were planted on February 5, 1930. The remaining species and varieties were planted one year earlier. All the plants were making satisfactory growth at the time of inoculation with a few exceptions, which will be noted. Most of the inoculations on these were made in May, 1930.

In consideration of the possible existence of pathogenic strains of the fire-blight organism, eight cultures were used for the majority of the inoculations. These represented pear, apple, Cotoneaster, and Pyracantha and, geographically, California, Michigan, New York, and South Carolina. They were composited immediately before they were used and no experi-

[1] Submitted for publication August 22, 1930.

[2] In the names of plants Rehder (5) is accepted as the guide for the most part.

[3] A typewritten thesis by Adrian C. Wilcox (Relation of miscellaneous plants to the pear industry. Thesis for the degree of Bachelor of Science in the University of California. May, 1920. Unpublished) on file in the Division of Plant Pathology of the University of California records successful inoculations on a single shoot each of *Pyracantha coccinea*, *Cotoneaster pannosa*, and *C. microphylla*.

ments designed to distinguish differences in pathogenicity between the cultures were undertaken. Inoculations were usually made from bouillon cultures with dissecting needles ground to a fine point.

The tips of growing shoots were inoculated in most instances. In a few cases in which these failed, later inoculations were made on older stems. Since none of the latter were successful, they will not be treated separately. In a single instance (*Cotoneaster Harroviana*) flower clusters were inoculated.

RESULTS OF INOCULATION EXPERIMENTS

It is highly desirable to place on record some evidence of the degree to which susceptible plants may be affected. This has been attempted in the results of some of the inoculations summarized in table 1. The data obtained from inoculations at any given date, however, are only approximately comparable to those of another date, though they be taken from the same planting and at short time intervals. Infection and the subsequent course of disease seem to be very intimately related to conditions of growth rate of the plant and to temperature and humidity during and after the incubation period. There is, moreover, the probability that certain plants may escape inoculation in nature more often than do others.

The problem of definitely placing a given species as to the degree of susceptibility is further complicated in the genera Cotoneaster and Pyracantha by the fact that considerable variation exists within certain species. This is not surprising in view of the common practice among nurserymen of growing these plants from seed. Specific instances of distinct differences in response to inoculation by plants of the same species from different sources will be noted later.

Infection experiments in the field probably afford a more nearly exact basis for predicting the response of plants to infection in nature than experiments in the greenhouse. The latter will be reported therefore only when some special interest attaches to them. Since more particular attention was given to the genera Cotoneaster and Pyracantha, these will be treated in somewhat greater detail.

Judging from parallel inoculations on a susceptible variety of apple (White Astrachan), it is concluded that the conditions under which the inoculations on plants of these genera were made were only moderately favorable for infection.

COTONEASTER MED. Several species of Cotoneaster have gained wide popularity as ornamental shrubs in California. There may therefore be considerable direct loss from blight, due to the death in whole or in part of the shrubs themselves, as well as a considerable menace to neighboring fruit trees. The results of inoculation experiments on the following fourteen species are presented in table 1.

Cotoneaster acuminata Lindl.
" *adpressa* Bois
" *Dammeri* Schneid. var. *radicans* Schneid. (F. P. I. 52677)[4]
" *Dielsiana* Pritz. var. *elegans* Rehd. and Wils.
" *Francheti* Bois
" *frigida* Wall.
" *Harroviana* Wils. (In part F. P. I. 72794)
" *horizontalis* Deene.
" *lactea* (F. P. I. 62569)
" *microphylla* Wall.
" *pannosa* Franch.
" *prostrata* Baker (F. P. I. 56304)
" *salicifolia* Franch. var. *floccosa* Rehd. and Wils. (In part F. P. I. 62256)
" *Simonsii* Bak.

For those species which were inoculated only in the greenhouse, the extent of invasion by the organism is not tabulated, since these tests are not considered comparable with those made in the field.

Three preliminary tests on a small scale were made with *C. adpressa*. In two of these a small amount of infection resulted. In a fourth test 20 shoots were inoculated on 2 plants. After 12 days, 14 of these were blighted. The organism penetrated to a maximum distance of about $1\frac{1}{2}$ inches from the point of inoculation but in most cases was checked at about $\frac{1}{2}$ to $\frac{3}{4}$ inch. All these tests were made in the greenhouse.

Two small plants of *C. Dammeri* var. *radicans* were available for greenhouse study. Five inoculations were made on each of two dates. Vigorous infection resulted in all of these and invasion was rapid until all the new growth was involved.

In addition to the field inoculations of *C. Francheti*, recorded in table 1, seedlings were inoculated in the greenhouse on four different dates. No symptoms developed on any of these.

Late in the season of 1929 at Chico, California, *C. frigida* was observed affected by blight in the larger branches as well as in the smaller shoots. An attempt to isolate the organism was unsuccessful, but the failure may be accounted for by the exceedingly dry weather in that region throughout the summer and autumn. From cuttings taken on the University campus at Berkeley a single plant was obtained in the greenhouse. Five days after inoculation distinct symptoms were evident on the plant and in 11 days all the new growth was involved. In striking contrast is the resistance

[4] The writers are indebted to the Office of Foreign Plant Introduction of the Bureau of Plant Industry, United States Department of Agriculture, for some of the plants used in these tests. The corresponding F. P. I. number is shown in such cases.

TABLE 1.—*Results of inoculation of Cotoneaster and Pyracantha with the fire-blight organism, Bacillus amylovorus*

Name of plant	No. of plants	No. of inoculations	No. of infections	Average distance traversed by organism (inches)[a]		Shoots invaded beyond current season's growth
				In 30 days	In 55 days	
C. acuminata	5	30	0			
C. adpressa	2	20	14	[b]		
C. Dammeri var. *radicans*	2	10	10	[b]		
C. Dielsiana var. *elegans*	5	30	0			
C. Francheti	5	30	0			
C. frigida	5	30	0			
C. Harroviana	5	30	2	[c]		
C. horizontalis	5	20	4	3.2	4.7	3
C. lactea	2	25	0	[b]		
C. microphylla	5	20	2	0.3	0.3	
C. pannosa	5	20	14	7.3	12.2	8
C. prostrata	2	20	1	0.5	0.5	
C. salicifolia var. *floccosa*	7	28	8	5.5	7.2	2
C. Simonsii	5	30	0			
P. angustifolia	5	20	13	5.6	7.5	9
P. coccinea	5	30	2	0.3	0.3	
P. coccinea var. *Lalandii*	5	20	5	3.4	3.4	
P. crenulata	5	20	12	5.5	7.4	6
P. crenulata var. *kansuensis*	5	20	12	5.8	7.8	7
P. crenulata var. *Rogersiana*	5	20	8	2.8	3.2	1
P. Gibbsii var. *yunnanensis*	5	20	4	8.8	11.0	4
P. formosiana	5	20	13	2.9	3.9	1
Apple (White Astrachan)	2	20	8	4.7	5.8	1

[a] Average from the shoots which actually became infected.

[b] Inoculated in the greenhouse, only.

[c] Both infections in flower clusters.

shown by the plants from another source inoculated at San José. The plants of this species from different sources vary considerably in appearance as well as in susceptibility to blight.

Plants of *C. Harroviana* were rather severely blighted in a garden at Berkeley in 1929 and the fire-blight organism was obtained from these in pure culture. Nevertheless, 13 inoculations on plants (F. P. I. 72794) in

the greenhouse on three different dates failed to produce any symptom of disease. It will be noted further that inoculations in the field on plants from another source produced at most a relatively mild infection. Here, apparently, is another case of variation as to susceptibility within a species.

PYRACANTHA ROEM. (FIRETHORN). The position of this genus in popularity among the ornamental shrubs in California is similar to that of Cotoneaster. The species and varieties used in these tests are listed below and the results of inoculations are presented in table 1.

Pyracantha angustifolia Schneid.
" *coccinea* Roem.
" " " var. *Lalandii* Dipp.
" *crenulata* Roem. (F. P. I. 55997)
" " " var. *kansuensis* Rehd. (F. P. I. 40736)
" " " " *Rogersiana* A. B. Jacks (F. P. I. 72814)
" *Gibbsii* A. B. Jacks var. *yunnanensis* Osborn
" *formosiana* (?)

Pyracantha angustifolia is one of the most attractive of the shrubs grown in California particularly during the winter months. Unfortunately, it also is one of the most susceptible species of this genus. Natural infections were abundant in Berkeley during the summer of 1930 and in one planting the disease was severe.

Pyracantha Gibbsii var. *yunnanensis* has exhibited rather marked variation in response to inoculations. It will be noted (Table 1) that the extent of invasion following inoculations in the field is greater than in any other species of the genus, although the number of infections was small. In contrast are the results of a small number of inoculations on seedlings from three different sources in the greenhouse. Although these inoculations were made on six different dates from January to July, the plants from two sources developed no symptoms, whatever. A single plant from the third source became blighted apparently as readily as did the plants in the field.

CHAENOMELES LINDL. Blighted fruits of *Chaenomeles sinensis* Koehne were received from a garden in Berkeley where scions of the species had been grafted on the common quince, *Cydonia oblonga* Mill. (These fruits were placed in a moist chamber and soon became entirely involved by the disease). The symptoms were typical of fire blight, which was also present on the shoots and blossom clusters of the common quince. The bacterial exudate from the fruits of *C. sinensis* was used to inoculate 5 seedling apple shoots. At the end of 4 days all of these bore typical symptoms of fire blight.

DIOSPYROS L. (PERSIMMON). In the first experiment with this genus, ten inoculations were made in the field on a single plant of *Diospyros Lotus* L. After 8 days all the inoculated shoots showed some blackening around

the wounds made by the inoculating needle. At the end of 14 days one shoot was blighted to a distance of 4 inches from the point of inoculation and bore a small amount of bacterial exudate. At the same time the discoloration had spread on 2 other shoots for a short distance ($\frac{1}{4}$ to $\frac{1}{2}$ inch). No further development of symptoms was noted, however, after this time. Thirty additional inoculations were made on shoots of this species at later dates. Only slight symptoms developed in 3 of these.

Diospyros Kaki L. was inoculated on two occasions in a total of 15 shoots. Some evidence of infection was seen on 2 shoots but this was slight and the species is consequently left in the doubtful class.

Thirty inoculations were made on *D. virginiana* L. without the production of any symptoms.

HETEROMELES ROEM. Waite (8) in 1906 observed the typical symptoms of fire blight on *Heteromeles arbutifolia* Roem., the common Toyon or Christmas berry, at Vacaville, California. This author states that "several of the twigs contained the living bacilli" but, apparently, he made no cultures or infection experiments. Although Waite's conclusion has been supported by the observations of a number of workers since that time, it has seemed desirable to make inoculations on this species. Seedlings were inoculated in the greenhouse on three dates. Infection resulted on each occasion, although the plants were small and not in so vigorous condition as is desirable. Eleven inoculations were made on 5 poorly growing plants in the field. Seven infections resulted, involving most or all of the new growth in each case. In view of all the evidence it cannot be questioned that this species may be and frequently is affected by fire blight in nature. The growth condition of the plants used in these experiments does not permit of any attempt to estimate exactly the degree of susceptibility of the Toyon as compared with the other plants studied.

KERRIA DC. *K. japonica* DC. was inoculated on three occasions in the greenhouse and once (on 20 shoots) in the field. No trace of a symptom resulted in any instance.

PHOTINIA LINDL. Pierstorff (4) in a paper now in manuscript records successful infection experiments with *P. villosa* DC. The writers have tested (in the field) *P. serrulata* Lindl. and a similar form sold under the name *P. dentata* for which they have found no authority. At the time of the first inoculations the plants were making rather poor growth. No infection resulted from 16 inoculations on each sort. Later when the growth condition of *P. serrulata* was somewhat better, 20 inoculations were made on this species and 5 on *P. dentata*. No certain symptoms were produced.

PRUNUS L. While fire blight has been recorded on several species of this genus, there is distinctly less susceptibility here than in the subfamily Pomoideae of the rose family. It is desirable, however, to make further

inoculations in this and related genera in order that a more nearly balanced view may eventually be obtained of the relative susceptibility of the plants which may be affected by the fire-blight disease. The complete list of Prunus species used in these tests is given below.

Prunus allegheniensis Porter	Alleghany plum
" *americana* Marsh.	Plum
" *Armeniaca* L.	Apricot
" *avium* L.	Cherry (Bing variety)
" *Besseyi* Bailey	Western sand cherry
" *bokhariensis* Royle	Plum (India)
" *cerasifera* Ehrh.	Myrobalan (cherry plum)
" *Cerasus* L.	Cherry (morello)
" *communis* Arcang.	Almond
" *dasycarpa* Ehrh.	Purple apricot
" *domestica* L.	Agen or French prune
" *hortulana* Bailey	Hortulana plum
" *ilicifolia* Walp.	Holly leaf cherry
" *insititia* L.	St. Julien G. and Black Damas C.
" *mahaleb* L.	Mahaleb cherry
" *mira* Koehne	
" *mume* Sieb. and Zucc.	Japanese apricot
" *Persica* Batsch	Peach (a flowering variety)
" *salicina* Lindl. X *P. cerasifera* Ehrh. (Myrobalan)	Methley plum
" *serrulata Lannesiana* (Carriere) Koehne	A flowering cherry
" *Simonii* Carr.	Apricot plum
" *tomentosa* Thunb.	Manchu cherry

Since relatively few infections were produced on the plants of this genus, the results are not presented in tabular form.

From 5 to 50 (usually ten) inoculations were made on each species. Parallel inoculations on apple demonstrated that the conditions at the time these were made were quite favorable for infection and more so than was the case with the inoculations on Cotoneaster and Pyracantha. In some instances effects were produced, such as gumming or discoloration at the point of inoculation, which may have been due to the presence of the organism, but only those species that exhibited more definite symptoms will be treated in the following paragraphs.

All of the inoculations with the exception of those on *P. ilicifolia* were made in the field on May 23, 1930, and the final notes were made on July 1.

Ten shoots of *P. allegheniensis* were inoculated. At the end of the experiment 5 of these bore some symptoms of blight. Invasion was very limited, however, varying from $\frac{1}{4}$ to $\frac{3}{4}$ inch.

Two varieties of apricot, Tilton and Royal, were tested. On the Tilton there was a small amount of discoloration and bacterial exudate but none of the tips of shoots was entirely killed. On the Royal variety 7 of the 10 inoculated shoots were killed at the tip or on one side of the shoot to distances of $\frac{1}{4}$ to $1\frac{1}{4}$ inches.

Ten shoots were inoculated on 2 plants of *P. Besseyi*. Nine of these suffered some injury, mostly less than 1 inch in extent. One of them, however, blighted to a distance of 3 inches in 11 days, accompanied by the typical bacterial exudate.

Fifty inoculations were made on 5 selections of myrobalan plums, 10 on each lot. On 2 of these lots, both of the red-leaf type, distinct symptoms were produced. One collection listed in the station records as Davis myrobalan 2567 yielded 8 infections ranging in extent from $\frac{1}{2}$ to $2\frac{1}{2}$ inches. The other, propagated from a chance myrobalan seedling, became slightly affected in 4 of the 10 inoculated shoots.

Prunus dasycarpa became infected in 3 of 10 inoculated shoots, with a maximum penetration of $1\frac{1}{2}$ inches.

About 30 inoculations were made on seedlings of *P. ilicifolia* in the greenhouse on 5 different dates. Following one of these there was some killing of the tips of shoots, but, since the results of all the other tests were entirely negative, it is concluded that these plants probably are highly resistant to, if not immune from, fire blight.

Two lots of *P. mume* were inoculated. On one of these, 2 of 10 inoculated shoots showed a small amount of killing but scarcely enough to more than place this species in the doubtful class.

The hybrid Methley plum developed 8 infections from 10 inoculations, with a maximum invasion of 1 inch.

Prunus Simonii became infected in 8 of 10 inoculated shoots, but the lesions ceased development at $\frac{1}{4}$ to $\frac{3}{4}$ inch in length. Paddock (3) observed what he believed to be natural infection on this species in Colorado, in 1903, but apparently made no cultures or inoculation experiments.

PYRUS L. (PEAR AND APPLE). *Pyrus Malus* L. var. *Niedzwetzkyana* Asch. and Graebn., a red-leaf, ornamental crab apple, was inoculated in 6 shoots. Invasion was slow but definite and finally resulted in killing on 4 shoots to an extent of $1\frac{1}{4}$ to $6\frac{1}{2}$ inches.

Five inoculations were made on a single young tree of *P. prunifolia* Willd., plum-leaf crab, in the field. Infection resulted in 3 shoots with a maximum invasion of 3 inches. In a later test, all of 10 inoculated shoots were blighted to a distance of $\frac{1}{2}$ to $3\frac{1}{4}$ inches from the point of inoculation.

The hybrid kaido crab, *P. micromalus* Bailey, was inoculated in 9 shoots. Only 2 of these became infected but these were eventually killed to 4 and 12 inches from the point of inoculation.

RAPHIOLEPIS LINDL. Two species of these evergreen shrubs were tested at San José.

Five plants of *R. indica* Lindl. in only moderate vigor were inoculated in 21 shoots. Twelve of these developed some symptoms and several were invaded for 2 to 5 inches. In view of the growth condition of the plants, it is concluded that a rather marked susceptibility inheres in this species.

The 5 plants of *R. umbellata* Mak. were small and made poor growth throughout the period covered by these experiments. Twenty-one inoculations were made on these. There was some evidence of invasion in a few shoots, but further inoculations are desirable before any definite conclusions are drawn for the species.

SPIRAEA L. Twenty inoculations were made on each of *S. cantoniensis* Lour., *S. prunifolia* Sieb. and Zucc., and *S. Vanhouttei* Zabel. No infection resulted. These inoculations were repeated later on the same plants. Some injury resulted on *S. Vanhouttei* and *S. cantoniensis* but it was scarcely sufficient to be conclusive. No symptoms were produced on *S. prunifolia*.

A blight of *Spiraea* sp. has been reported as fire blight from Maryland (1) (without experimental proof), and Rosen and Groves (6) reported symptoms of blight resulting from inoculations on detached twigs of this species.[5]

DISCUSSION

An exact account of the degree to which susceptible plants under natural conditions may be affected by fire blight must await more extensive observations, even for plants which have long been recognized as affected by this disease. For example, apricot, cherry, plum, and prune have been on record for many years as susceptible, yet few observations seem to have been made on fire blight among these plants in the orchard. Relatively little time has been given by the writers to a search for natural infection of the susceptible plants here reported for the first time. Such infections have been seen, however, presenting the typical symptoms of fire blight on *Cotoneaster frigida*, *C. Harroviana*, *C. pannosa*, *Pyracantha angustifolia*, *P. coccinea Lalandii*, *P. crenulata*, *P. crenulata kansuensis*, and *P. crenulata Rogersiana*. The fire-blight organism was isolated from blighted specimens of 3 of these species.

It is inferred by comparison with parallel inoculations on apple that *Cotoneaster pannosa*, *Pyracantha angustifolia*, *P. crenulata*, *P. crenulata kansuensis*, and *P. Gibbsii yunnanensis* exhibit a degree of susceptibility approaching that of apple and pear. The limited tests with *C. Dammeri radicans* indicate a rather marked susceptibility in this species.

[5] Since this paper was written, Groves has presented conclusive evidence of the susceptibility of *S. Vanhouttei*. (U. S. Dept. Agr. Plant Dis. Rep. 14: 133. 1930).

It is probable that the blight organism will be found to survive the winter in plants of several of these species, since cankers not uncommonly extend into branches several years old. The menace to fruit trees, however, offered by these shrubs, is not so great as it may appear, since most of the susceptible species of Cotoneaster and Pyracantha come into blossom distinctly later than the pear and apple. This is also true of the Toyon.

The existence of even a slight degree of susceptibility in a genus so remotely related to the rose family as Diospyros (ebony family) suggests that the list of susceptible plants may yet be far from complete. It is of interest, however, to note that within a single susceptible species (*Cotoneaster frigida*) or variety (*Pyracantha Gibbsii yunnanensis*) plants may be found that are highly resistant to if not immune from fire blight.

SUMMARY

Fifty-six species, representing eleven genera of plants, were inoculated with the fire-blight organism, *Bacillus amylovorus*. Some evidence of the degree of susceptibility of plants in certain of these species is presented.

Among the species of Cotoneaster, *C. Dammeri radicans*[6]*, *C. horizontalis**, *C. pannosa**, and *C. salicifolia floccosa** are distinctly susceptible. *Cotoneaster adpressa**, *C. microphylla** and *C. prostrata** may be infected but exhibit a rather marked resistance, while *C. acuminata*, *C. Dielsiana elegans*, *C. Francheti*, *C. lactea*, and *C. Simonsii* have remained unaffected through these experiments. Some plants of *C. frigida** and *C. Harroviana** are distinctly susceptible, while others are not. This appears to be due to variation within the species.

*Pyracantha angustifolia**, *P. crenulata**, *P. crenulata kansuensis**, *P. crenulata Rogersiana**, *P. Gibbsii yunnanensis**, and *P. formosiana** are at least moderately susceptible, while *P. coccinea* and *P. coccinea Lalandii** are somewhat less so.

Symptoms were produced by inoculations upon plants of *Prunus allegheniensis**, *P. Armeniaca*, *P. Besseyi**, *P. cerasifera**, *P. dasycarpa**, and *P. Simonii**, but all of these species appear to be relatively resistant.

Evidence is presented to show that *Chaenomeles sinensis** may be affected by fire blight in nature.

Successful infection experiments were made upon plants of *Diospyros Lotus**, *Heteromeles arbutifolia*, *Pyrus Malus Niedzwetzkyana**, *P. micromalus**, *P. prunifolia**, and *Raphiolepis indica**.

[6] The species and varieties for which no experimental proof of susceptibility has been found in the literature are marked by an asterisk.

The range of those plants that may be affected by fire blight is shown to be in need of further intensive and extensive study.

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RYE INFECTED WITH BUNT OF WHEAT

E. N. Bressman

During the past season two varieties of rye were inoculated with bunt, *Tilletia tritici* (Bjerk.) Winter and *T. levis* Kühn, from wheat and infection was obtained. In 1923 Gaines and Stevenson[1] found bunt on rye at Pullman, Washington. *T. secalis* (Cda.) Kühn has long been known in Europe, according to Kühn.[2]

Other workers have reported bunt on rye. Kirby,[3] in regard to *Tilletia tritici* on rye, says: "The stinking smut of rye has been reported but once in New York. Rye is very resistant to it, and never more than a few infected heads have been found in a field. The stinking smut in wheat commonly found in this state, is caused by another species of smut fungus. Whether this fungus ever attacks rye is unknown."

In 1928 Johnston[4] stated: "In Kansas several thousand heads of rye grown from seed heavily inoculated with bunt were examined and three heads were found which were bunted. The rye was a mixture in badly smutted wheat seed and plants grown from that seed were heavily smutted."

In the last 3 years the writer has received 100 collections of bunt from various American and foreign sources. Sixteen of these collections were placed on spring rye sown at Corvallis in the fall of 1928. None of these infected the spring rye. In the fall of 1929, 26 of the outstanding collections of bunt obtained from the variety Hybrid 128 grown at Corvallis were placed on Oregon Rye Sel. and 5 collections on pure Rosen rye obtained from the Michigan Agricultural College.

The seed was heavily coated with bunt spores from the various collections and sown in rod rows in the cereal nursery at Corvallis. One collection of *Tilletia tritici*, classified as physiologic form 9, in work to be published later, was the only one that infected rye at Corvallis. Six heads of bunt, or 6.1 per cent, were found in 98 heads of Oregon Sel. 1, and 4 heads of bunt, or 3.5 per cent, were found in 115 heads of Rosen rye. None was found in either the checks or rows inoculated with other forms of bunt. There were about 4,000 heads of rye in these trials.

[1] Gaines, E. F., and F. J. Stevenson. Occurrence of bunt in rye. Phytopath. **13**: 210–215. 1923.

[2] Kühn, J. *Tilletia secalis*, eine Kornbrandform des Roggens. Bot. Ztg. **34**: 470–471. 1876.

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FIG. 1. Normal and smutted heads of rye. A. Normal Oreg. Sel. 1 (note sterility). B. Smutted Oreg. Sel. 1. C. Normal Rosen. D. Smutted Rosen. Corvallis, Oreg. 1930.

The collection of bunt that infected rye was obtained from Morrow County, Oregon, an important wheat-growing section. It is one of the most virulent strains on wheat obtained by the writer and infects many of the so-called resistant varieties.

In duplicate material from Pendleton, Oregon, grown and counted for the writer by J. Foster Martin, 133 heads of bunted rye were found in the 2 varieties. Oregon Rye Sel. No. 1 was inoculated with 15 collections of bunt and Rosen rye with 5 collections in the Pendleton trials of which there were about 6,000 heads. Both species of bunt produced infection. Al-

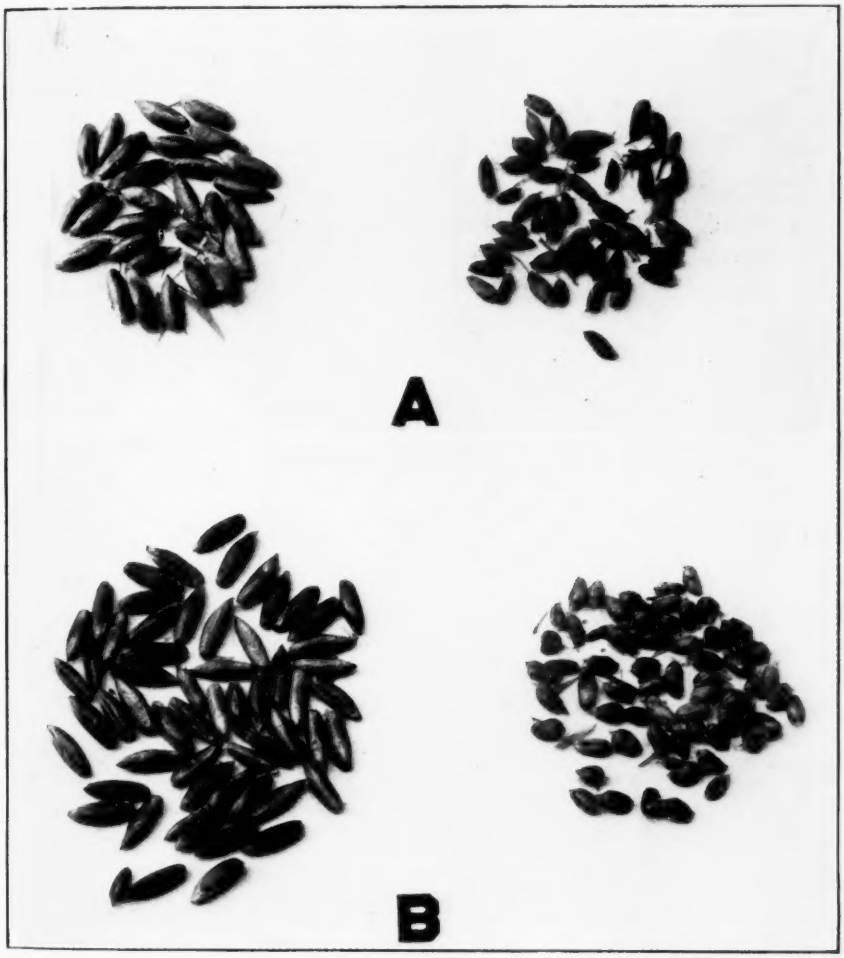

FIG. 2. Normal and smutted kernels of rye. A. Normal kernels and bunt balls of Oreg. Sel. 1. B. Normal kernels and bunt balls of Rosen. Corvallis, Oreg. 1930.

though several collections of bunt infected rye at Pendleton, physiologic form 9 gave the highest percentage.

Figure 1 shows normal and bunted heads of both varieties. It shows no evidence of dwarfing of the bunted heads of these 2 varieties.

The number of bunt balls found in the diseased heads of the Oregon rye is of interest. There is, ordinarily, considerable sterility in this variety and many of the flowers do not produce seed. The diseased heads produced bunt balls in practically all florets. Figure 2 shows typical normal kernels and bunt balls from the 2 varieties and gives an idea of the number of bunt balls formed in comparison with the number of grains in a typical head.

The bunt produced on rye at Corvallis had the typical odor of wheat bunt. The spores averaged about 19 microns in diameter and were typical of *Tilletia tritici*. This material is being used in additional trials to determine definitely whether or not *T. secalis* is a form of *T. tritici* and should be designated as such.

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PHARMACIEN FRECHOU AND THE GERMINATION OF SCLEROSPORA OOSPORES

WM. H. WESTON, JR.

It was in 1878 and 1879 that *Sclerospora graminicola* (Sacc.) Schroet. was first reported from Italy, from France, and from Germany, the first accounts of its morphology and development were presented, and its status as the type species of a new genus was established superseding tentative affiliations with Peronospora or Protomyces. The work done by the first investigators of this fungus, chiefly Saccardo, Passerini, and Schroeter is familiar to all. These pioneers did not secure germination of the resting spores even though Schroeter, realizing the importance of working out this phase of the life history, attempted it repeatedly. In the activity which followed calling attention to this fungus other investigators persevered in the endeavor to fill in the gaps in its life history and in 1884 Prillieux reported M. Frechou's success in germinating the oospores.

Since these early years of investigation this fungus has been found on various grasses (chiefly Setarias), both cultivated and wild, in many parts of the world and many of the problems which it involves have been studied by mycologists and plant pathologists. The resting spores, consisting of the single oospore closely enveloped in the thickened, golden to chestnut colored oogonial wall, have been subjected to morphologic and cytologic study, have been used as inoculum in infection tests, and on good evidence have been assumed to be the means by which the fungus survives unfavorable conditions, persists from season to season, and spreads from country to country.

Yet, during almost half a century of such investigation, no further cases of oospore germination were reported and nothing was added to the meagre knowledge of this phase of the life history.

Now, however, within the past year or two, in a recrudescence of activity in the study of this downy mildew, Hiura[1] not only has reported his own successful germination of resting spores of the fungus from cultivated millet in Japan and, presumably from the same host, in the United States, but also has mentioned seeing these bodies successfully germinated by DaNami; while, recently, Evans and Harrar,[2] working under Melhus, report having repeated the procedure successfully with oospores from

[1] Hiura, M. A simple method for the germination of oospores of Sclerospora graminicola. Science, n. s. **72**: 95. 1930.

[2] Evans, M. M., and George Harrar. Germination of the oospores of Sclerospora graminicola (Sacc.) Phytopath. **20**: 993–998. 1930.

foxtail grass, *Setaria viridis*, at Ames, Iowa, and give the first published drawings of the process.

These papers, unfortunately, do not clear up the confusion that exists among various references in mycological literature to the previous germination of *Sclerospora graminicola* oospores. Gäumann,[3] for example, in his useful and authoritative "Vergleichende Morphologie der Pilze," p. 81, after the statement that germination of the oospores takes place "bei Sclerospora und Peronospora durch ein Keimschlauch, der in der Wirtspflanze zu einem Mycel auswächst" gives deBary 1865 as authority but it is to be feared, without adequate grounds, while Dodge in his extensively revised translation hardly improves the situation by adroitly omitting reference to deBary, thus leaving the statement unsupported. Fitzpatrick in his accurate and comprehensive account of the "Phycomycetes" refers to the method of oospore germination as undetermined. Hiura refers to Magnus as the investigator who succeeded previously in germinating the oospores, while Evans and Harrar point out that there have been confusions and uncertainties and errors in such references without helping to clear up the situation.

In the abundant literature on *Sclerospora graminicola*, from the time of its discovery until the recent work just noted, there is, as far as the writer, is aware, but one reference to any observation of the germination of these resting spores. This, as noted above, is contained in a contribution read by Prillieux[4] before the Societé Botanique de France, séance of December 14, 1884, and published in the Bullétin of that Society. In this, M. Prillieux reports that M. Frechou, pharmacist in the village of Nerac in the Midi of France, in the preceding year had called to his attention *Peronospora setariae* (*Sclerospora graminicola*) on *Setaria verticillata*, and from his own observations and those of M. Frechou, he describes accurately the season and nature of development of both the conidial and oosporic phase of the fungus. He then continues, "Les oöspores, ou spores d'hiver, apparaissent vers le mois de septembre, en grand nombre, à l'intérieur des feuilles; elles sont globuleuses, jaunâtres, ont une paroi épaisse et lisse, et sont contenues dans des oögones ovales ou globuleux à paroi mince. Ces spores dormantes germent au printemps suivant. M. Frechou les a vues émettre un tube comme celles du *Peronospora viticola*, mais n'a pu poursuivre au delà l'étude de leur developpement. Il est donc encore incertain si l'oöspore du *Peronospora setariae* peut produire directement un stipe conidiophore, comme je l'ai annoncé pour le Peronospora de la Vigne."

[3] Gäumann, E. Vergleichende Morphologie der Pilze. Gustav Fischer. Jena. 1926.

[4] Prillieux, E. Sur le Peronospora setariae. Bul. Soc. Bot. France. **31**: 397–398. 1884.

This descriptive note, even though scanty and without any supporting illustrations, none the less carries a conviction that germination of the oospores actually was witnessed and it apparently is the basis of the general impression that the oospores germinate by germ tubes in a manner somewhat resembling that of Peronospora or of the grapevine mildew.

It is rather surprising that this lone account of oospore germination in this fungus should have escaped attention so completely. To be sure, the writer,[5] thinking that it might be of interest to others working in this field, referred to it in a discussion of "Production and Dispersal of Conidia in the Philippine Sclerosporas of Maize" in 1921 (p. 273), but even so it remained unnoted either because readers were overcome with fatigue before they had reached this point in that somewhat lengthy paper or because in that sentence the word "paramacien," one of the five unscotched typographical errors in the paper, so intrigued them with its possible protozoan connotations that the potentialities of the reference itself escaped them.

Yet this neglected report of 1884 still has certain mycologic and historic interest. As far as we know, it is not only the first description of the method of oospore germination in *Sclerospora graminicola*, but also the only description of this process during nearly fifty years' investigation of this and other species of the genus. Moreover, in revealing this tantalizing glimpse of Monsieur Frechou, Prillieux's report possesses a certain human interest. Ce pharmacien, que diable allait-il faire dans cette galère? Who was this obscure investigator whose significant observation transmitted unostentatiously through Prillieux has been credited to famous mycologists such as deBary and Magnus? One pictures him, a quiet man with an observing eye and seeking mind, on whose time the duties of compounding a few simple prescriptions for the village made no excessive demands, retiring to the corner of his orderly and aromatic little shop to look through his treasured microscope at diatoms as well as drugs, at moth-wing scales and mildews, at pollen grains, and Peronospora spores. One imagines him in the cool of the evening sitting in his garden and over a bottle of wine discussing with his friend Prillieux questions and problems in the science dear to them both. Pharmacien Frechou, the first to work out the germination of Sclerospora oospores, an accomplishment which it has taken mycologists fifty years to repeat,—what other interesting observations did he make that have, alas, been lost to us? Any further knowledge of him and of his work would, indeed, be welcome.

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[5] Weston, Wm. H., Jr. Production and dispersal of conidia in the Philippine Sclerosporas of Maize. Jour. Agr. Res. 23: 239–278. 1923.